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SELECTED ARTICLES—

ORIGINAL ARTICLES

"KUMRI" (SECOND PROGRESS REPORT)

BY

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INTRODUCTION

MATERIAL for investigations forming the subject-matter of this progress report was obtained from seven cases of "Kumri" sent to the laboratory from different parts of the province. The animals were kept at the laboratory under observation and treatment for variable periods and then destroyed humanely under chloroform. Careful *post mortem* examination was held in each case and tissues were removed and preserved for histopathological studies. Clinical observations, results of the examination of blood, urine, faeces and the cerebro-spinal fluid and *post mortem* findings are incorporated in this report, while the correlation of the histopathological findings is held in abeyance as the greater part of this work on the central nervous system still remains to be done.

CLINICAL OBSERVATIONS

The cases under investigation were fairly representative of the disease in various stages. All the animals were "aged". Out of 7 cases—one was an Arab ; 4 were Walers and 2 were country-bred. Two were entires ; 3 were geldings and 2 were mares. They were received from the following places :—

Name of Place	Number received
Arrah	1
Purnea	2
Hazaribagh Police Training College	2
Muzaffarpur	1
Bettiah	1
	7 Animals

In all cases paresis of both the hind legs (paraplegia) was present. This was shown by the characteristic stance—the paretic hind legs being held wide apart and the fore legs being carried backward under the body to bear more than their share of the body-weight and by the marked inability to back without dragging their toes along and tending to fall. The paraplegia in every animal was of the spastic kind (spasm and rigidity), there being neither flaccidity nor wasting of the muscles of the limbs showing loss of strength. In almost all the cases, it was noticed, that one hind limb, usually the near hind, was more affected than the other. It was further observed, that in the untreated cases the paresis was distinctly progressive.

The gait in each case was that of spastic paraplegia combined with ataxy. The spastic element in the gait was shown by the dragging of the hind limbs slowly forward with the toes scraping the ground, very little flexion of hocks, pronounced circumduction, specially noticed during a trot when the legs were swung outward in an attempt to clear the ground, and the hind quarters showing the characteristic swinging and strong adduction spasm, so that the legs both in walking and trotting tended to cross each other. In two cases, the spastic element was, indeed, so pronounced, that the gait actually assumed a "hopping" character. The ataxic element of the gait was evidenced by the fact that, during walking, the hind feet were lifted hesitatingly and incompletely from the ground, as though in an attempt to correct the imperfect muscular sense. This imperfect muscular sense was clearly seen in backing—all the animals showing great lack of confidence, dragging their toes along, and tending to fall when backed quickly. The ataxic element of the gait was particularly well brought out, when the animals were made to turn; they lifted their limbs very cautiously, invariably crossed them, and tended to fall when turned rapidly.

Two cases showed distinct tremor of muscles on attempting to move somewhat similar to the volitional or so called "intention tremor" seen in insular sclerosis. The tremor was most marked in the hind legs. It was absent, when the animals were at rest, and practically unnoticed during slow walk, but whenever the animals were made to trot, a course of jerky twitching of the muscles, frequently convulsive throwing up of the hind legs, invariably occurred.

In no case was any loss of sensation observed. On the contrary, all cases showed lumbar hyperalgesia, as evidenced by the visible wincing and crouching caused by pressure over the loins. As regards the vesical and rectal sphincters, in no case were they found to be affected.

Examination of faeces

Repeated microscopic examination of the faeces and scraping from selected areas of intestinal mucosa was carried out. Almost all cases showed eggs of round worms but the ova of schistosomes were not encountered.

Examination of urine

Chemical and microscopical examination of the urine was carried out on several occasions. In all advanced cases the urine was invariably bright yellow in colour, and its consistency was such that it poured like thick linseed oil. On standing, a thick yellowish somewhat flaky sediment settled down at the bottom of the flask. Chemical examination showed that this turvidity was entirely due to carbonates and excessive amount of phosphates. This yellowish colour and thick oily consistency, with the characteristic sedimentation on standing, appeared to be so striking and invariable a feature in all advanced cases, that it might prove very helpful in the diagnosis of the disease. It was repeatedly found, that during the improvement resulting from the specific therapy advocated by this laboratory, there was a noticeable change in this character of the urine. Chemical examination failed to reveal any other abnormality. Microscopic examination showed crystals of carbonates and triple phosphates in abundance, and in each case, cylindroids were present. Repeated examination for schistosome ova gave negative results.

Examination of the blood

Repeated examination of blood smears from each case failed to reveal any parasites. No corpuscular abnormalities, *e.g.*, anisocytosis, poikilocytosis, polychromasia, or nucleated red cells were met with. Each case, however, showed a slight but distinct increase in the number of eosinophiles, and this was proved by the differential count, which showed the eosinophiles to be 14-16 per cent of the total number of leucocytes (normal for the horse being 3-4 per cent.) Hœmoglobin estimations always gave a figure in the neighbourhood of 85 per cent.

Clotting of blood

Tests, similar to those described in my paper on Nasal Granuloma [1932], were carried out on a few samples of blood to find out the retractability of the clot in cases of "Kumri", which appeared to have an identical etiology. It was found that there was a similar non-retractibility of clot in these samples as was observed in the samples obtained from cases of Nasal Granuloma.

Formol-gel test (aldehyde reaction) with serum

While experiments were being carried out on the retractability of the clot, Formol-gel tests were applied to many samples of serum with a view to excluding the possibility of protozoan parasites (Leishmania and trypansomes) lurking in the system of the animal. The results were all along negative.

Cerebro-spinal fluid

In view of the fact that some workers have reported the presence of filaria in cerebro-spinal fluid in cases of "Kumri", this fluid was obtained from four cases by the method usually adopted for the intrathecal injection of Naganol

in surra, and each sample carefully examined for protozoan and helminthic (schistosomes and filaria) parasites. The results were in all cases negative. The fluid was found to be clear in all cases except one, in which the turbidity was obviously due to accidental admixture with blood. Examination of the cellular content of the fluid by Thoma Zeiss counting apparatus revealed, in three cases, only a "slight mononucleur Pleocytosis". Two of the four samples showed traces of glucose, while the other two failed to show any glucose when tested with Fehling's reagent. Pandy's test gave, in two cases, an "Opalescence" showing the presence of globulin. No quantitative examination of albumen could be carried out, but qualitative examination by Aufrecht's method showed about equal amount of albumen in all the four cases.

POST-MORTEM FINDINGS

All the cases at the laboratory were humanely destroyed by chloroform and careful *post mortem* examinations were carried out. The abnormalities noted in connection with the different parts were as follows :—

Alimentary canal.—In view of the fact that existing literature contains no records of habronema tumours in the stomach in cases of "Kumri", mention must be made here of the finding of these tumours, varying in size from that of a golf ball to that of a billiard ball, in four out of seven cases. In fact, these tumours were so constantly found in the first few cases that they appeared to have some etiological relationship with the disease. The position of habronemiasis had, however, so many distinct points of divergence from "Kumri" that it was ruled out of consideration in connection with etiology—a position which has later on proved to be correct by the absence of such tumours from other three cases. Incidentally, reference must also here be made to the chemotherapeutically important fact that full course of intravenous injections of antimony compounds had produced no effect whatsoever on these worms. In two cases the stomach contained gestrophilus larvae. Apart from these findings the stomach did not show any other pathological change. No abnormalities were noticed in the small intestines, and the only notable, although by no means unusual, feature in the large intestine was the presence of a variable number of nematodes, chiefly strongyles, sclerostomes and trematodes.

Mesenteric vessels.—In view of the fact that the symptom-complex of parasitic aneurysm of the anterior mesenteric artery shows some points of resemblance to that of "Kumri", special pains were taken to make careful search for such aneurysms in all cases. Only in one case was such an aneurysm present. Schistosomes were, however, encountered in the blood, obtained in the manner described in the previous report, from the mesenteric vessels of three animals, that had not been treated, and in the one case, that had died after the third injection. The mesenteric vessels were congested in all cases.

Peritoneum.—Nothing abnormal was noticed in connection with the peritoneum, or its contents. In only one case a filarial parasite (*Setaria-labiato-papillosa*) was met with, but this case showed neither ocular filariasis nor filarial nodules in the liver.

Liver.—In all cases, the capsule of the liver showed patchy fibrous adhesions to the parenchyma. Careful search was made, in the manner described in my previous report, for schistosomes in the portal vessels. Out of these seven cases four had been treated and three untreated. In all the untreated cases schistosomes were recovered from the portal blood. Out of the four treated, three had been treated with antimony compounds and one with emetine hydrochloride. Schistosomes were found in the animal treated with emetine and in the animal, which died after the 3rd injection with tartar emetic, but no schistosomes could be found in others treated with antimony compounds. Some portions of the liver, in a few cases, showed distinct cirrhotic changes.

Iliac Vessels.—Another disease, the syndrome of which may simulate that of "Kumri", is iliac thrombosis. Attention was accordingly focussed on these vessels in each case, but no evidence of thrombosis was present. It may here be mentioned that rectal exploration on the living animals had been carried out in some cases, and this too had failed to reveal the presence of such thrombosis.

Spleen.—The spleen did not show any abnormality. Only one case showed a slight congestion of the renal medulla. The suprarenals were apparently unaffected, and nothing noteworthy was seen in connection with the bladder, heart, lungs, pleural contents, lymphatic glands, the skeletal structures or muscles. In one case cysts were present in the ovary.

Nervous system.—The brain and the entire spinal cord of each animal were laid bare by carefully sawing through the bones of the skull and vertebral canal, and then carefully removed by cutting through their connections with the skull and vertebrae. In these exposed cavities, search was, at this stage, made for filaria and schistosomes, and many smear and cover slip preparations from different parts were examined for protozoa and helmenthic ova. The results of this examination have, so far, been negative.

In each case the olfactory lobe, the cerebellum, the cerebrum, the caudate nucleus, the hippocampus and the medulla oblongata were cut into and thoroughly searched for abnormalities. In no case were filaria or schistosomes encountered, and in no case was there any evidence of macroscopic change, either in the meninges or the substance of the brain. In one case only were the vessels of the choroid plexus rather significantly tortuous. Smear and cover slip preparations from different parts have so far failed to reveal any protozoa or helmenthic ova.

In the different regions of the spinal cord also, close searching, combined with cover slip and smear examination, failed to reveal any protozoa, helminths or their ova. In two cases gelatinous infiltration was observed around the cord in the dorsal and lumbar regions. In no case was there, however, any macroscopic evidence of patchy or leptomeningitis. Even in the cases in which the lumbar region of the cord showed disorganization, there was no morbid alteration of the dural, arachnoid, and pial membranes.

The morbid anatomy of the substance of the cord, as revealed by close inspection of its cut surface, appeared to vary according to the stage at which the animal was destroyed. In less severe cases, and specially those that had responded to treatment, the grey matter did not show anything beyond a few punctate haemorrhages, specially in the lumbar region of the cord. In more advanced cases there was a distinct vascularity of the grey matter giving it a pink appearance. In the case which was clinically very advanced, and which gave no response whatsoever to specific therapy with compounds of antimony, the lumbar portion of the cord appeared to have had undergone such disorganisation that the line of demarcation between the grey and white matter was rendered quite indistinct.

The white matter did not show any marked naked eye abnormality in five cases. In two other cases (Bhutani and Arrah Black) the white matter of the first part of the lumbar cord showed, specially in the vicinity of posterior cornuas, small pea-sized tumour-like growths. Only one such tumour was present in the Bhutani, while the Arrah pony showed two, one near each cornua of the grey matter. The surface of the cord opposite to that containing the tumours showed in each case a cavity in which the tumour was apparently lodged in the intact cord. Histological examination, now in progress, will reveal whether these growths are neoplasmatic gliomas, or merely the result of hypergliosis.

Discussion

Clinical observations detailed in this report have revealed sufficient uniformity in the syndrome to justify regarding "Kumri" as an entity. The paraplegia with the elements of ataxy and spasticity, the absence of muscular wasting of trophic and sensory disturbances and the non-affection of rectal and vesical sphincters are seen in each case. Many workers have written about the difficulties of diagnosis in cases of "Kumri". Series of observations have shown that wherever with the symptom complex, usually described, one gets the characteristic thick sedimenting urine, rich in phosphates, and showing cylindroids on microscopical examination, one can be pretty certain that one is dealing with a case of "Kumri."

The results of the chemical and microscopical examination of the cerebrospinal fluid would appear to show some points of correspondence with the syndrome met with in human disseminated sclerosis, in so far as the slight cell increase and

the weak globulin reactions are concerned. It remains to be seen whether Lange's Colloidal Gold reaction will yield a " paretic " type of Lange's curve, and whether quantitative examination of the protein will reveal little or no excess, for, then the correspondence between the two diseases would be exact—the Wasserman reaction, the absence of which is of diagnostic value in disseminated sclerosis, being ruled out of consideration, when dealing with equines, naturally insusceptible to *Treponema pallidum.*

Post mortem examinations have revealed the presence of numerous schistosomes in the liver in every untreated case of the disease, and blood examination has also revealed a slight but distinct eosinophilia, the occurrence of which while common to many helminthic infestations and dermatic disturbances, is quite a notable feature in schistosomiasis.

Conclusion

(1) " Kumri " is a definite morbid entity, because the majority of affected animals present a uniform symptom-complex.

(2) Schistosomes are present in numbers in the liver in every case of " Kumri ".

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A PRELIMINARY MICROPHOTOGRAPHIC STUDY OF THE FAT GLOBULES OF THE MILK OF INDIAN BREEDS OF COWS AND BUFFALOES

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(With Plates I-VI)

Introduction

The size, shape and number of fat globules of milk are of practical importance in the separation of milk, the churning of cream, the shipping of milk or cream over long distances, in cheese-making and in the manufacture of other products of milk. The size of fat globules bears a relationship to the churning of cream as the cream with larger fat globules churns with less agitation than that in which the smaller globules predominate. The size is of further importance in connection with the handling and shipping of milk. Milk having large globules churns more rapidly and when transported over long distances may show evidence of partial churning in the form of butter granules on the surface, which makes the milk unsuitable for marketing purposes.

Most of the Western breeds of cattle have been studied and classified according to the suitability of their milk for different dairy products like butter, cheese, milk, etc., depending on the size, shape and number of fat globules in it. For example, the Channel Island breeds, (*e.g.*, the Jersey and the Guernsey) are known as "butter breeds" due to the fact that besides containing a higher percentage of fat their milk contains fat globules of large size, which makes them very suitable for easy churning and consequently for butter-making. Similarly of all the British breeds the Short Horn and the Ayrshire are considered more suitable for cheese-making because of their milk having fat globules of medium size and fairly uniform in shape. The Indian breeds of cattle had not, however, been systematically studied so far from that point of view and this work was, therefore, undertaken by the authors to ascertain whether by the study of the size, shape

and number of fat globules in the milk of various Indian breeds of cows and buffaloes, a similar classification could be made which would help the dairyman in the choice of the breed of cow or buffalo most suitable for his requirement.

Early history of the detection of fat globules in milk.—The presence of fat globules in milk was first reported by A. Van Leewenhoeck in 1674. The manner in which fat globules of milk are liberated by the mammary gland was explained by Marshall [1922] in the form of three hypotheses which he put forth.

Bouchardat and Quevenne [1857] observed that the size of the fat globules varied appreciably in the milk of different breeds, and indicated that the globules in cow's milk varied from 10 to 2 microns in diameter.

Sturtevant [1873] reported the milk globules to appear as circles of unequal diameters, and stated that these globules were enclosed in a thin membrane or pellicle of extreme tenuity.

Babcock [1885] did not agree with Sturtevant that the fat globules were enclosed in a thin membrane or pellicle. He compared the globules in milk with artificial emulsions and concluded that they were not enclosed in a membrane but were surrounded by a thin film of serum.

Methods employed for the study of size, shape and number of fat globules present.—Different methods were used by different workers in the field for measuring the fat globules of milk. The method used by Lamson [1888] was to dilute the milk with three to four hundred parts of water, place a drop on a cover glass, and invert over a cell on an ordinary microscope slide.

Collier [1891-92] used the method developed by Babcock, and studied the variation in the size of fat globules in milk throughout the lactation period of individual animals.

Various other workers have studied the variation in the size of the fat globules in milk of individual animals of different breeds during their period of lactation, a more recent publication in the field being that of H. Campbell [1932].

Results of the microphotographic study of the fat globules in samples of milk of goats, cows, human beings and homogenised milk, have very recently been published in an American journal by Apple [1935].

EXPERIMENTAL

Names of breeds studied.—The following is the list of breeds of Indian cows and buffaloes which were considered for the purposes of this study. It represents breeds of cattle of the purely draft type as well as milch type. The list is not as complete as it should be and the omission of important breeds like the Sahiwal,

Amrutmahal, etc., although unintentional was unavoidable due to lack of proper response from the sources from which the samples were to be obtained :—

Milch breeds	Scindi. Gir. Tharparkar. Hariana. Kankrej. Ongole. Krishna Valley.	Draft breeds	Kangiam. Assamese. Malvi. Dhani.
		Buffalo ,,	Murrah. Surti. Nagpuri.

Line of study.—The main line of study was of the number, size and shape of the fat globules in the milk of the above breeds of cattle with a view to ascertain the differences in (*a*) the milk of cows and buffaloes, (*b*) the milk of milch breeds and draft breeds and (*c*) the classification of milch breeds according to the suitability of their milk for marketing, butter and cheese-making, etc., if possible.

Method of obtaining the samples.—The milk samples of the various breeds were obtained from different parts of India under the following strict constant conditions :—

1. Four samples—one pound each—were received on four successive days.

2. Each sample was representative of the herd and not from one individual animal.

3. Each sample, before being packed and despatched, was kept in as cool a condition as possible; and preserved by adding about six drops of formalin to a pound of milk.

4. Each sample was received in an ordinary glass bottle, securely packed, care being taken to fill the milk right up to the top to avoid air bubbles and to prevent fat globules churning or splitting during transit.

5. Each sample was labelled with the name of the breed, the number of milch animals in the herd (the average number being 42), the time of drawing of milk sample (which was always during early morning) and the date and the time of despatch.

The above conditions were adopted after carrying out preliminary experiments in order to obtain the samples in such a manner as not to damage or alter the size or shape of the fat globules. Various preservatives were tried in order to get the best results, and formaldehyde, which though commonly known to

be detrimental to the fat globules when used in fairly large quantities, was found to be most suitable. The minimum quantity of formalin required for keeping the milk fat globules in their original condition for even upto a fortnight, was found by experiments to be about six drops to a pound of milk.

The above conditions were found to be most practicable, since the samples received from such distant places as Baluchistan, Punjab and Assam were in the most satisfactory condition for the purposes of this experiment.

The experimental work was carried out during the first quarter of the year during which time the summer had not advanced so far as to have very harmful effect on the samples during transit.

Method employed in taking the photographs.—The photographs of the fat globules of various samples were taken with a vertical compact apparatus made by Messrs. E. Leitz, Watzler, which consists of a microscope, fitted with a camera to take pictures of the magnified image directly on to the negative plate in the camera. This apparatus is illustrated in Plate I.

A reference has already been made in this paper [Lamson, *loc. cit.*] to the various methods used by the different workers for this study. The following was the method adopted by the authors. After the proper mixing of the sample to get a representative picture, a drop of milk was taken on a cover glass, which was then inverted over a cell of a microscope slide, and a photograph taken directly after necessary focussing and the study of the preliminaries of the apparatus. The negatives of the camera were further magnified, and directly a print was taken.

Methods employed in counting and calculating the number and size of the fat globules.—The counting of the number of fat globules was carried out with the help of the counting apparatus devised by Jeffer. The ruled glass plate used was 165 mm. square with a circular ruled area 132 mm. in diameter. This area was divided by concentric circle into equal areas, which were subdivided by radii or segments of radii into smaller integral portions. The average number of fat globules given in Table I, represents a mean value obtained from counting about twelve photographic pictures in the case of each breed.

The magnification in all cases was 350, determined by means of a haemacytometer cell, and each picture given in Plates II-VI, Figs. 1 to 16 represents a constant original area of 0·0363 square mm. with the diameter as 0·21 mm.

The results of these experiments are given in Tables I—III.

Number, size and shape of fat globules in the milk of the breeds of Indian cattle

TABLE I

N.B.—In this table the names of the breeds are arranged in groups as classified into milch and draft breeds and buffaloes. The cross-bred and Ayrshire cattle have been included as the samples of their milk were available.

Breed		Number of fat globules			Diameter of globules of normal size (in microns)
		Average	Minimum	Maximum	
Milch Breeds.	Scindi cows	1,000	970	1,100	3·4
	Gir cows	1,203	910	1,540	3·1
	Tharparkar cows	1,140	1,045	1,376	3·4
	Hariana cows	1,090	1,013	1,177	3·4
	Kankrej cows	1,247	917	1,470	2·9
	Ongole cows	1,133	953	1,310	3·1
	Krishna Valley cows	1,078	992	1,352	2·9
Foreign Breeds.	Half-bred cows	1,002	803	1,126	3·4
	Ayrshire cows	1,066	1,013	1,177	3·7
Draft Breeds.	Kangiam cows	1,267	1,260	1,270	3·7
	Assamese cows	1,160	1,001	1,417	2·9
	Malwi cows	1,110	990	1,238	2·9
	Dhanni cows	1,073	953	1,200	2·9
Buffalo Breeds.	Murrah buffaloes	945	836	1,093	5·7
	Surti buffaloes	890	723	970	5·4
	Nagpuri buffaloes	712	560	790	5·7

TABLE II

In this table the breeds have been arranged according to the number of the fat globules found in their milk.

Breed	Number of fat globules		
	Average	Minimum	Maximum
Kangiam cows	1,267	1,260	1,270
Kankrej cows	1,247	917	1,470
Gir cows	1 203	910	1,540
Assamese cows	1,160	1,001	1,417
Tharparkar cows	1,140	1,045	1,376
Ongole cows	1,133	953	1,310
Malwi cows	1,110	990	1,238
Hariana cows	1,090	1,013	1,177
Krishna Valley cows	1,078	992	1,352
Dhanni cows	1,073	953	1,200
Ayrshire cows	1,066	1,013	1,177
Half-bred cows	1,002	803	1,126
Scindi cows	1,000	970	1,100
Murrah buffaloes	945	836	1,093
Surti buffaloes	890	723	970
Nagpuri buffaloes	712	560	790

TABLE III

In this table the breeds have been arranged according to the size of the fat globules present in their milk.

Breed	Diameter of globules of normal size (in microns)	Globules of normal size				Four globules of biggest size*			
Murrah buffaloes .	5·7	5·7	5·7	5·7	5·7	11·0	10·0	9·0	8·6
Nagpuri buffaloes .	5·7	5·7	5·7	5·7	5·7	11·4	11·4	10·0	9·2
Surti buffaloes .	5·4	5·4	5·4	5·4	5·4	10·0	9·2	8·6	8·6
Ayrshire cows .	3·7	3·7	3·7	3·7	3·7	7·1	7·1	7·1	6·3
Kangiam cows .	3·7	3·7	3·7	3·7	3·7	11·4	11·4	11·0	10·0
Half-bred cows .	3·4	3·4	3·4	3·4	3·4	7·1	6·3	6·3	6·3
Scindi cows . .	3·4	3·4	3·4	3·4	3·4	10·0	7·3	7·1	7·1
Hariana cows .	3·4	3·4	3·4	3·4	3·4	10·0	7·1	7·1	7·1
Tharparkar cows .	3·4	3·4	3·4	3·4	3·4	6·3	6·3	5·7	5·7
Ongole cows . .	3·1	3·1	3·1	3·1	3·1	6·3	6·3	5·7	5·7
Gir cows . .	3·1	3·1	3·1	3·1	3·1	6·3	5·7	5·7	5·7
Dhanni cows .	2·9	2·9	2·9	2·9	2·9	5·7	5·7	5·7	5·7
Krishna Valley cows	2·9	2·9	2·9	2·9	2·9	5·7	5·7	5·7	5·7
Malwi cows . .	2·9	2·9	2·9	2·9	2·9	6·3	5·7	5·7	5·7
Assamese cows .	2·9	2·9	2·9	2·9	2·9	6·3	5·7	5·7	5·7
Kankrej cows .	2·9	2·9	2·9	2·9	2·9	8·7	7·1	7·1	7·1

* *N.B.*—1. It was not possible to measure the size of very small globules which occurred as mere spots on the photographs.

2. In the case of the Ayrshire breeds the cows numbered two, so the size of the fat globules cannot be taken as accurate for purposes of comparison with other breeds whose number averaged 42.

DISCUSSION

It can be seen from the above results that there is no direct relationship between the number of fat globules, their size and the total percentage of fat in the milk samples. Still, it can be explained in a general manner that in samples containing a high number of fat globules the size of the fat globules was comparatively small and *vice versa*.

It can also be observed from the results that in the case of the buffaloes the size of the fat globules was on the whole large and the number consequently low as compared to the cows. This can be seen from Plate IV-VI.

Furthermore, it can be observed that the size of fat globules in the case of draft breeds is rather small compared to the fat globules in the milk of milch breeds. This can be seen from Plates II and III. Another interesting observation is that when the fat globules are medium in size they are fairly uniform in shape.

CONCLUSION

From the above observations it can, therefore, be concluded that :—

(1) Compared to the cow's the buffalo's milk contains fat globules of a larger size though smaller in number. For that reason buffalo's milk can be considered more suitable for butter and ghee manufacture and less suitable for marketing purposes when it comes to long distance transport.

(2) Of the two classes of cow breeds, *i.e.*, the draft and the milch breeds, the milk of the draft group contains fat globules, medium in size, more in number and more uniform in shape compared to the milch breeds, but as the draft breeds as a rule give very little milk in their present state of development, this quality of their milk cannot be put to much practical use.

(3) In the milch breeds themselves from the above study there does not appear to be any marked variation in the number, size and shape of the fat globules, as such it is difficult to classify these breeds for specific purposes, such as for market milk, butter-making, cheese-making, etc. But in practice and with years of experience in the actual handling of the milk of various important breeds it has been found that milk of breeds like the Gir, Sahiwal, Tharparkar, is pre-eminently suited for butter-making and therefore for ghee-making. Whereas, the milk of breeds like the Scindi, Kankrej, Ongole and Hariana is suited for marketing purposes, *i.e.*, selling milk as milk.

ACKNOWLEDGMENT

The authors take this opportunity of thanking the Live-stock Experts of different provinces and States and also the Provincial and State Directors of Agriculture for their kind co-operation in supplying the milk samples in the required condition.

The detailed examination of the samples was for the most part carried out in the well-equipped laboratories of the Department of Bio-chemistry of the Indian Institute of Science, and the authors are also thankful to the authorities for their kind co-operation.

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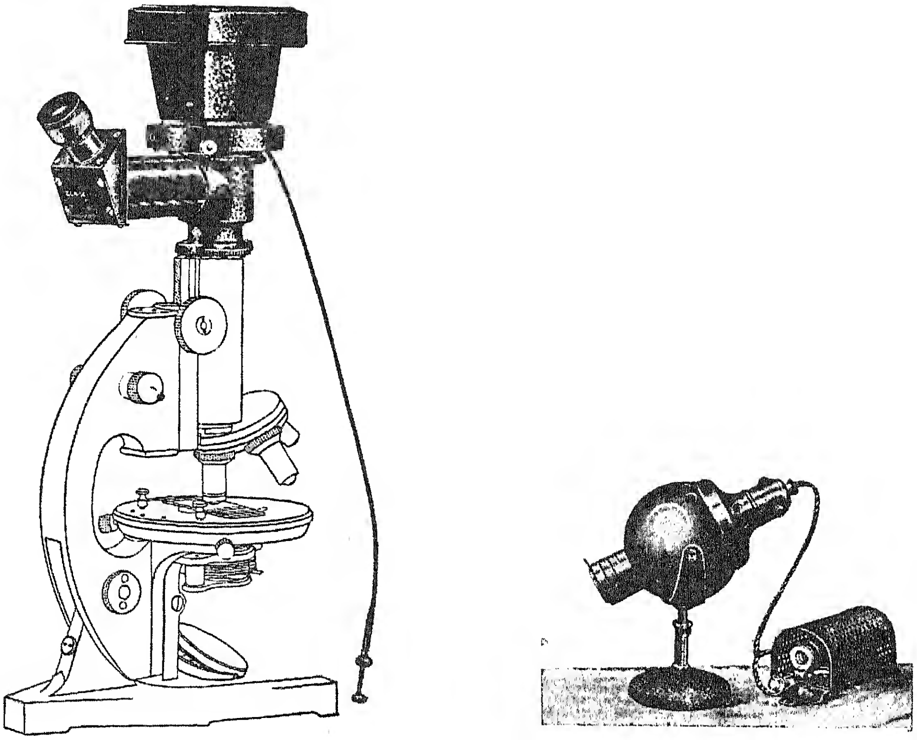
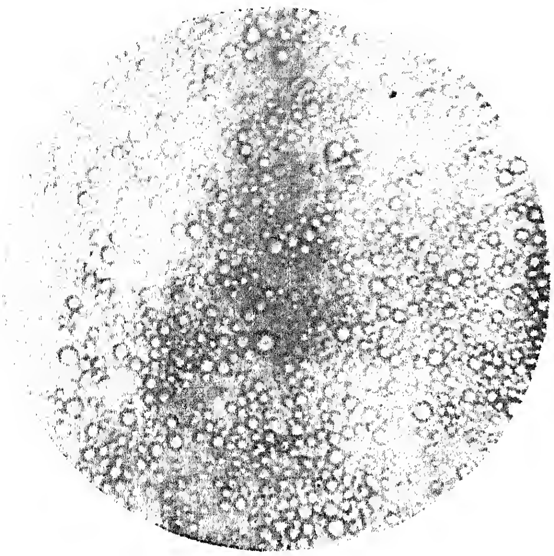


Compact outfit manufactured by Messrs. E. Leitz, Watzler for taking direct micro-photographs.

MICROPHOTOGRAPHS OF FAT GLOBULES IN MILCH BREEDS OF COWS.



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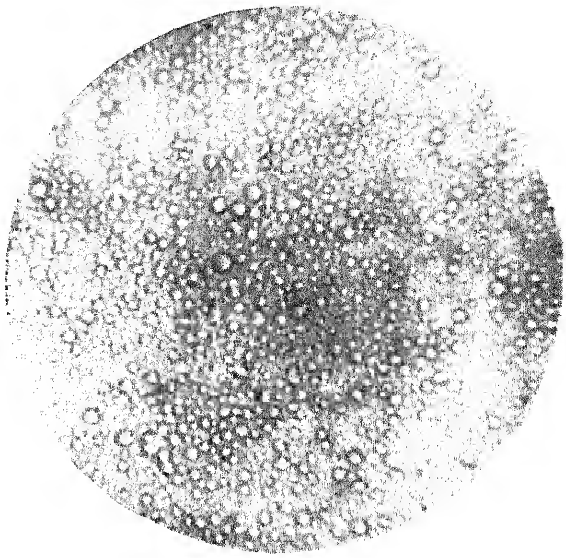

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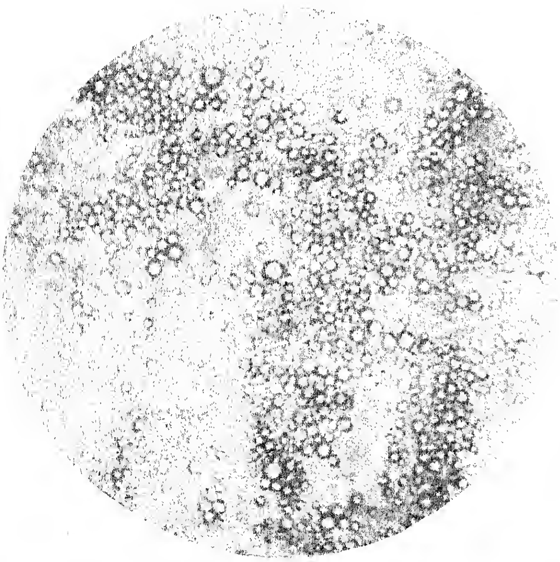

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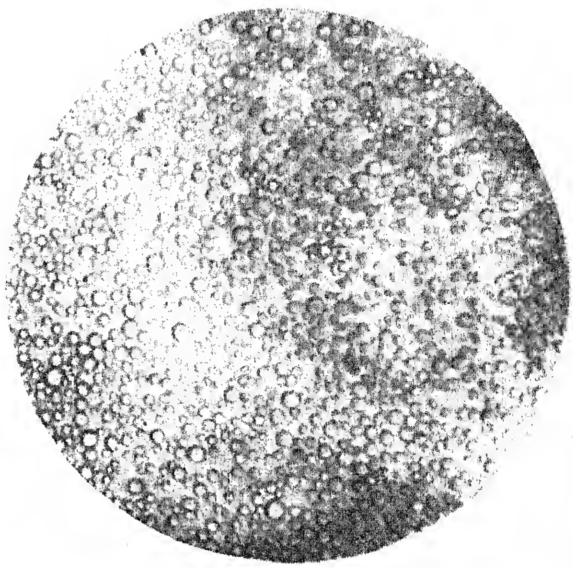

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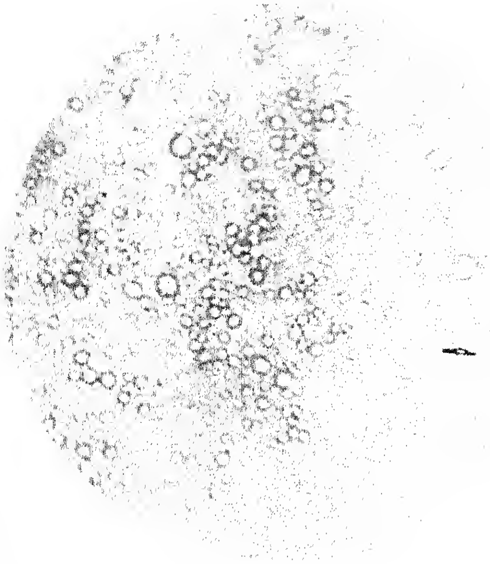
MICROPHOTOGRAPHS OF FAT GLOBULES IN MILCH BREEDS OF COWS.



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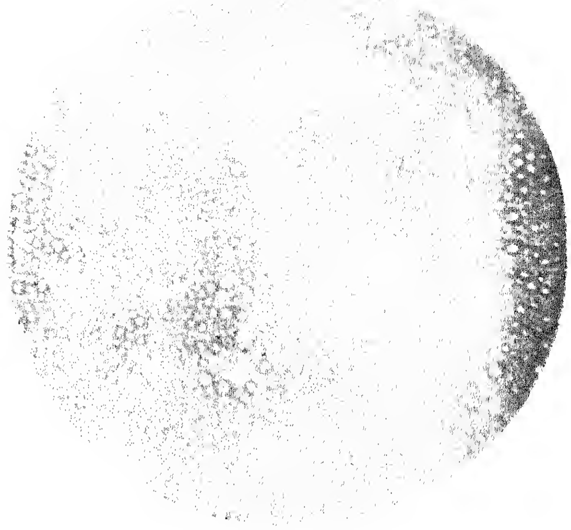

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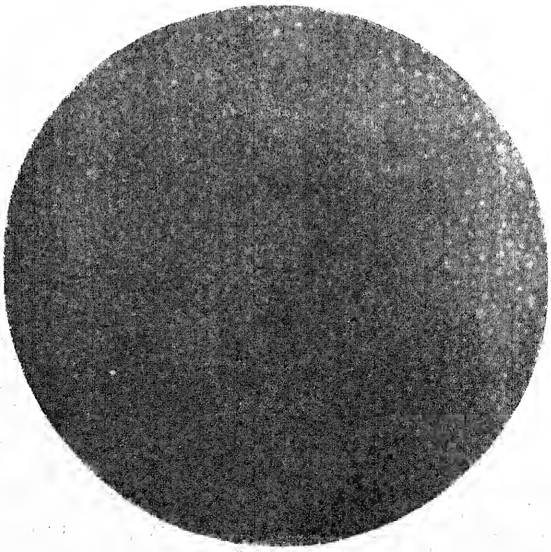

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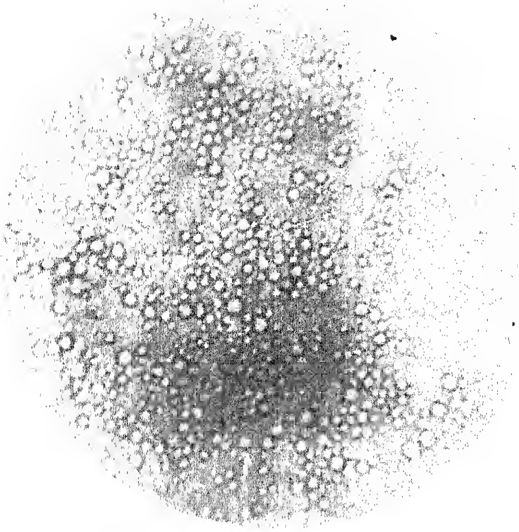
MICROPHOTOGRAPHS OF FAT GLOBULES IN BREEDS OF COWS.



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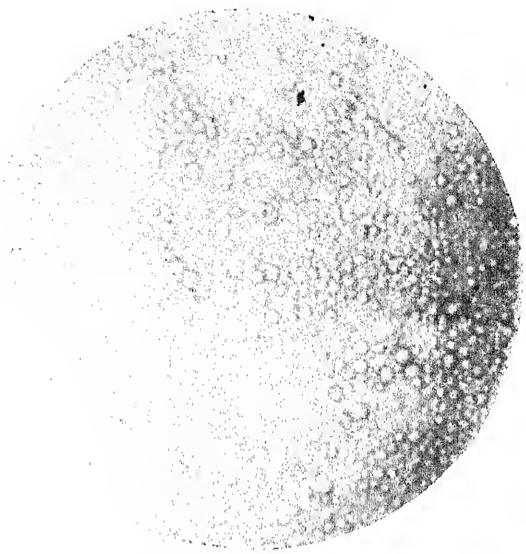

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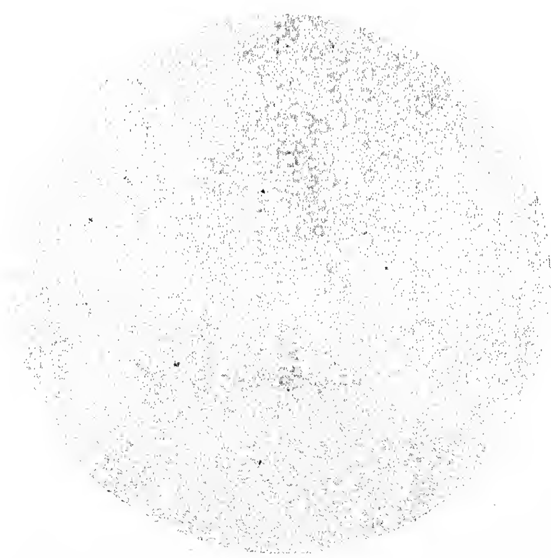
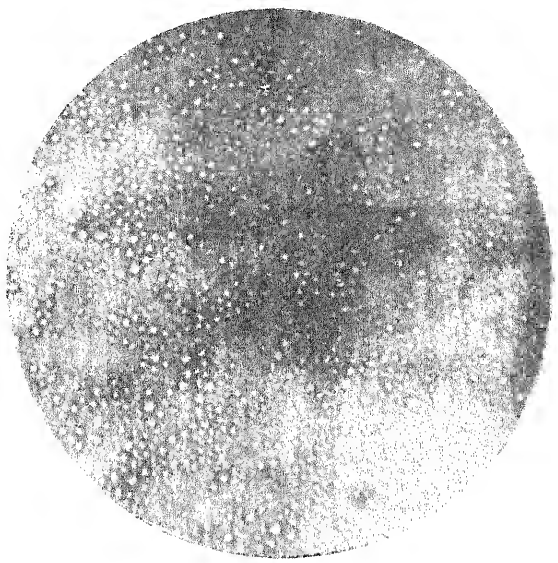


No. 10. Kangiam (Draft Breed)

MICROPHOTOGRAPHS OF FAT GLOBULES IN DRAFT BREEDS OF COWS.



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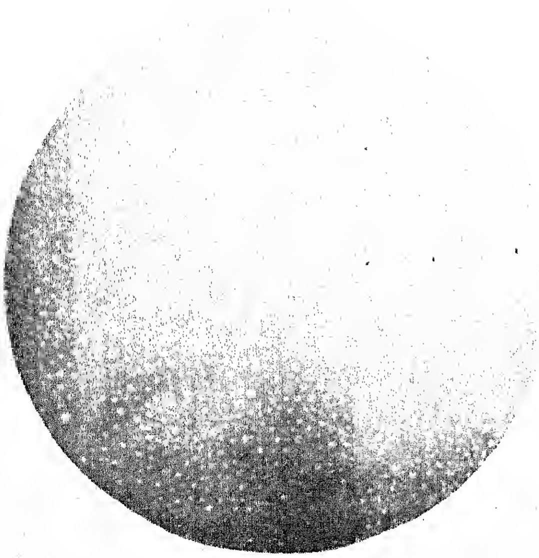
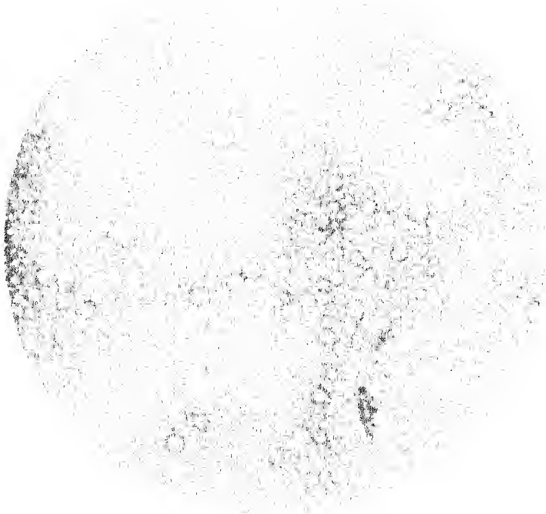


No. 12. Malvi

No. 13. Dhanni

MICROPHOTOGRAPHS OF FAT GLOBULES IN MILCH BREEDS OF BUFFALOES



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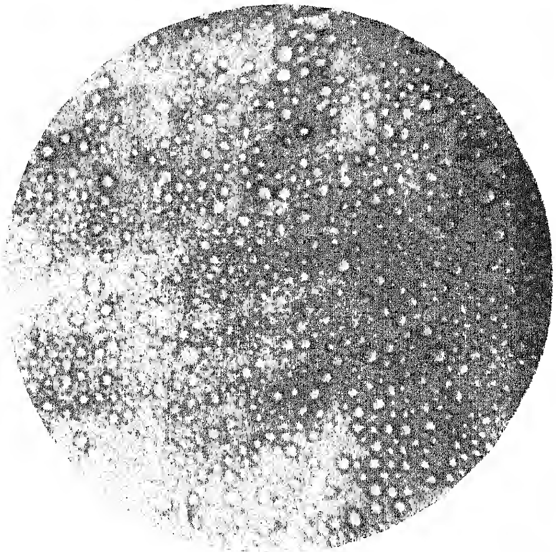

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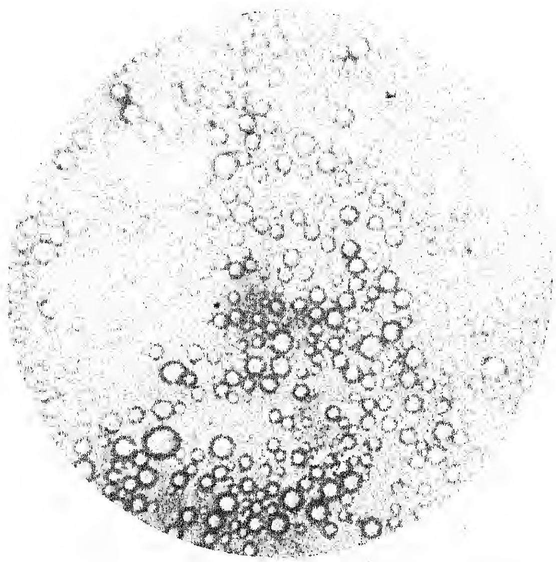

No. 16. Nagpuri Buffalo

THEILERIASIS OF CATTLE IN INDIA

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(With Plate VII and three text-figures).

CONTENTS

Introduction

Bovine theileriasis, as a condition distinct from redwater (*Babesia bigemina* infection) of cattle, was first recognized in India in 1925, when Edwards reported the occurrence in hill bulls of a species of protozoan parasite conforming with the

description of *Theileria mutans*. Previous to this, Theileria parasites in India used to be designated as "small piroplasms", as distinguished from *B. bigemina* or "large piroplasms", the presence of any of these forms in blood smears being held to "indicate infection with the causative agent of tropical 'red-water'" [Edwards, 1925]. About the same time, Cooper, working at this Institute, discovered certain structures, indistinguishable from Koch's blue bodies, in the lesions of bulls that had died after inoculation with rinderpest virus, these lesions presenting a resemblance to those of East Coast Fever, the blood picture in these fatal infections having been later characterized by Edwards [1930] as follows: "Almost 100 per cent of the cells were invaded, often with numerous parasites, and in some cases the elongated bacillary forms of piroplasms were frequent or even preponderated, instead of the oval form usually found almost exclusively in the latent type of infection". Structures, similar to those discovered by Cooper, have since been not infrequently encountered in this country, both in the blood and organ smears from acute cases of natural theileriasis in cattle, the parasite concerned in all such cases having been believed to be *T. mutans*, which, as is well known, is of widespread occurrence in the blood of cattle in India. The conditions under which this species of parasite, which is normally innocuous for the bovine species, attains the status of a virulent variant for these animals have been the subject of speculation, and the view has been tentatively advanced that the parasite might "gain special pathogenic properties in the body of a highly susceptible host" [Edwards, 1925], this possibility being suggested by the fact that Brumpt, in Paris, succeeded in exalting an Algerian strain of *T. mutans* by passaging it through French cattle that were normally free from infection with this parasite. It would appear that this was the only position that could be taken at the time concerning the identity of the parasite, particularly in view of the fact that all attempts to transmit the disease experimentally were consistently negative, this being held to be suggestive of the existence of a "carrier" infection in the majority of the cattle population in this country.

Since the foregoing observations were published, however, the position in regard to bovine theileriasis in different parts of the world has passed through a series of vicissitudes and the problem has now attained a degree of complexity which is perhaps not shared by any other protozoan disease of animals. In India itself, a mass of new facts has come to light necessitating a revision of the view previously advanced in regard to the specific identity of the parasite involved in fatal cases of theileriasis in cattle. One salient fact that has emerged out of the controversy that has centred round the question of the identity of these parasites is the unreliability of their morphological features as an indication of the extent of their pathogenicity. As pointed out by Sergent and his collaborators [1927], the actual pathognomonic element in theileriasis is the presence of the plasma bodies, so that the question of the specific identity of the theileria parasites themselves assumes a position of only secondary importance. On the other hand,

recent evidence would appear to point to the conclusion that even these bodies possess but a limited qualitative significance from the standpoint of pathogenesis, in view of their association, although in "extremely rare" numbers [du Toit, 1930*a*] with *T. mutans*, which, as already mentioned, is regarded by common consent as a harmless species.

A number of biological properties have been postulated from time to time as capable of being utilized for the differential diagnosis of the various species of Theileria parasites, but with the acquisition of more intimate knowledge concerning these properties, they have been found to possess but little specific value. Thus, the causal agent (*T. parva*) of East Coast Fever, which at one time had been believed to be non-inoculable with blood, has now proved to be definitely, although with difficulty, transmissible in this manner, whilst Bevan [1924] has even advanced the theory of latency, in an unrecognised form, of *T. parva* parasites in a proportion of recovered cases, although it has been an almost established opinion that the immunity developed after recovery from *T. parva* infection is both solid and sterile. A similar view has also been more recently expressed by Walker [1930], who reports having obtained evidence of the fact that "recovery from an original attack of *T. parva* did not confer a solid and lasting immunity". Finally, the status of *T. parva* itself, as the causal agent of East Coast Fever, has been questioned by Richardson [1930], who would seem inclined to the view that the condition is in reality induced by an ultra-visible virus.

It would thus appear that it is the quantitative combination of a number of dissimilar elements that is now held to be diagnostic of the different species of Theileria parasites. Thus, as Richardson [1930] points out, there is "little to distinguish between *T. mutans* and *T. parva* except the more numerous blue bodies and parasites in the latter infection". Furthermore, referring to certain blood smears sent by Cooper from India and showing numerous blue bodies, Richardson remarks that "had they been encountered in Africa, they would have led to the diagnosis of East Coast Fever".

The systematic side of theileriasis has thus assumed the aspect of a comparative science, with a considerable amount of scope for the expression of individual opinions, and this makes it a matter of difficulty to reach an objective decision on the several issues involved, particularly in regard to the relationship of one form of Theileria parasite to another. This is strikingly illustrated in the discordant views held on the subject. Thus, while, according to Carpano [1930], Tropical Piroplasmosis, East Coast Fever and *T. dispar* infection are all one and the same condition and caused by *T. annulata*, Van Saceghem [1924], on the other hand, regards *T. parva* as the only "good" species and *T. dispar* and *T. mutans* merely forms of this. Doyle [1924], however, disputes the identity of *T. mutans* with *T. parva* and considers the latter as being limited to Africa south of the equator

and *T. mutans* as being responsible for the forms of theileriasis occurring in Egypt, Sudan, Transcaucasus and the Mediterranean littoral. The unique practice of classifying the disease in different categories in accordance with the name of the locality in which it is known to occur, (*e.g.*, " East Coast Fever ", " Egyptian Fever ", " Mediterranean Coast Fever ", " Tropical Piroplasmosis ", etc.), further illustrates the difficulty of adopting the conventional method of designating the condition on the basis of the specific identity of the causal organisms.

It would seem that a considerable amount of further data will have to be acquired in regard to the nature of the problem of theileriasis itself before its solution can be attempted on a rational basis, and it is mainly with the object of contributing towards that end that the present article has been written. It is hoped that the observations embodied herein will be found to contain some new matter for consideration, particularly in relation to pathogenesis of the disease and the phenomenon of immunity.

The present paper deals with work carried out, during the period from June 1932 to August 1934, with a strain of Theileria parasites isolated from a fatal case of the disease in a hill bull at Muktesar. The strain is still being kept alive by passaging, but the observations carried out after August 1934 mainly concern a method of treating the condition with anti-serum and they will form the subject of a second communication.

The paper also includes, at relevant points, brief references to the more important outbreaks of theileriasis that have occurred during recent years among Friesian bulls imported into India for the use of the Military Dairy Department.

It is our pleasant duty to express our grateful thanks to Mr. F. Ware, F.R.C.V.S., I.V.S., Director of the Institute, for much helpful advice and guidance and for keeping us constantly alive to the importance of this investigation. In our account of the outbreaks of theileriasis among imported bulls, we have freely drawn from the observations recorded by Dr. J. T. Edwards, the late Mr. Hugh Cooper, Captain S. C. A. Datta and Mr. P. R. Krishna Iyer, and for this we are greatly indebted to them. Our thanks are due to Mr. Sunder Rao and Mr. Ahmed Baksh, Artists, for preparing the illustrations.

Source of strain

Previous to the work described in this paper, several attempts had been made to transmit the disease experimentally to hill bulls, but, as already mentioned, these attempts had been unsuccessful. On going over the records of these trials, it seemed to us that the failure to transmit the disease in this manner might have

been due to the fact that the infective material had lost its viability by the time it was used for sub-inoculation into experimental animals. In the present trials, therefore, the material for sub-inoculation was derived from an animal that was destroyed *in extremis* at a stage when its blood showed Theileria parasites to the extent of about 1 to 10 in each red blood-corpuscle, associated with fairly large numbers of Koch's blue bodies found both free and in the large mono-nuclear leucocytes.

Oboldouef and Galouzo [1928], in experimenting with a species of Theileria, designated by them *T. turkestanica*, used emulsions of gland materials and defibrinated blood for purposes of subinoculation. On the other hand, Walker and Whitworth [1929], in Kenya, in the course of their experiments upon the artificial immunization in East Coast Fever, observed that the disease could be transmitted not only by the inoculation of blood and gland juice, but also of spleen pulp, this last having yielded the largest percentage of successes. The infective materials used in our experiments comprised, in addition to the three mentioned above, emulsions of liver, kidney and lung. The quantity of infective material used ranged from 100 to 200 c. c. and the inoculations were given by the intravenous route, one bull being used for each type of inoculum. The results obtained in the case of the two bulls which received the liver and lung emulsions were of an entirely negative order, but all others developed symptoms of acute theileriasis as a result of the infective inoculation, and in three out of these four animals the disease progressed to a fatal issue (it is, however, noteworthy that material derived from liver has since proved infective).

The batch of four reactors mentioned above formed the starting point of the present inquiry, the strain being carried on from these and maintained to this day in hill bulls by blood inoculation. An injection of 5 c. c. of blood usually resulted in infection, but, in order to ensure against the strain being lost, the quantity actually used as a routine procedure was 20 to 30 c. c. administered subcutaneously, the blood being drawn at the height of thermal reaction, when the parasites, as also Koch's bodies, appeared in fairly large numbers in the peripheral circulation, although in a few cases the disease was also successfully reproduced by the inoculation of blood drawn at the height of temperature but containing only few parasites and apparently no Koch's bodies.

In all, forty-four sub-passages, involving the use of 273 bulls, have been carried out (Table I) without any evidence of the strain having undergone either attenuation or exaltation in virulence. Nearly 47 per cent of the animals proved refractory to the infection, whilst the mortality rate amongst those that developed the disease as a result of infective inoculation amounted to nearly 76 per cent.

TABLE I

Showing the number of sub-passages carried out through hill bulls and their results

No. of passages	No. of bulls used for each passage	No. of reactors in each passage	Average incubation period in days	Deaths	Recoveries	Immune or non-reactors	Remarks
I . .	*6	4	14	3	1	2	*Infective materials from the original (natural) case.
II . .	6	3	16	3	..	3	
III . .	2	1	16	..	1	1	
IV . .	4	3	19	3	..	1	
V . .	4	4	15	4	..	..	
VI . .	4	4	19	2	2	..	
VII . .	4	1	16	1	..	3	
VIII . .	6	3	16	2	1	3	
IX . .	6	3	16	3	..	3	
X . .	6	3	18	3	..	3	
XI . .	8	4	16	2	2	4	
XII . .	10	6	15	6	..	4	
XIII . .	6	3	15	3	..	3	
XIV . .	4	2	18	1	1	2	
XV . .	8	1	18	1	..	7	
XVI . .	8	5	14	5	..	3	
XVII .	6	6	16	4	2	..	
XVIII .	9	5	18	3	2	4	
XIX . .	10	5	15	4	1	5	
XX . .	6	4	15	4	..	2	
XXI . .	6	3	14	2	1	3	
XXII .	6	3	16	3	..	3	
XXIII .	6	2	15	2	..	4	
XXIV .	6	4	16	3	1	2	
XXV .	6	2	16	1	1	4	
XXVI .	6	4	17	4	..	2	
XXVII .	6	3	15	2	1	3	
XXVIII .	6	1	19	..	1	5	
XXIX .	6	4	17	4	..	2	
XXX. .	7	3	16	2	1	4	
XXXI .	6	3	14	1	2	3	
XXXII .	6	3	16	..	3	3	
XXXIII .	6	2	15	..	2	4	
XXXIV .	6	2	20	2	..	4	
XXXV .	6	2	16	2	..	4	
XXXVI .	6	1	23	1	..	5	
XXXVII .	12	6	18	3	3	6	
XXXVIII .	6	2	17	1	1	4	
XXXIX .	5	4	16	3	1	1	
XL . .	6	3	16	2	1	3	
XLI .	6	6	16	4	2	..	
XLII .	6	6	15	6	..	..	
XLIII .	6	2	16	2	..	4	
XLIV .	6	4	15	3	1	2	
	273 bulls	145 or 53·1 per cent	16 days (average of 145 reactors)	110 or 75·86 per cent	35 or 24·14 per cent	128 or 46·9 per cent	

Host susceptibility

All experimental evidence points to the conclusion that the parasite is specific to cattle. Its infectivity was tested upon two buffaloes, two sheep, two goats and a rabbit, but the results were negative.

Incubation period

The period of incubation of the disease, *i.e.*, the time intervening between the infective inoculation and the appearance in the circulating blood of either the parasites or Koch's bodies or both, associated with an elevation of the body temperature, was found to be 13 to 23 days, the average of about 145 reactors being 16 days.

Symptoms

The first noticeable symptom of the disease is an elevation of the body temperature, which may be to the extent of 3 to 4 degrees or more above the normal, this being accompanied by a running from the eyes, mouth and nostrils. The animal, however, continues to feed well during the first two or three days after the onset of these symptoms, but as the disease advances it develops progressive inappetence, until eventually it goes completely off its feed. At the beginning of the pyrexial period, the faeces are very hard, but diarrhoea soon sets in, and when the disease runs a prolonged course, the evacuations are frequently mixed with blood and mucus, although the urine usually remains unchanged. By this time, the animal becomes greatly emaciated and assumes a recumbent position. In a majority of cases, the prescapular and precrural glands are enlarged and tender, whilst the visible mucous membrane of the eyes shows varying degrees of icterus and petechiae. In a proportion of cases (Fig. 1), the temperature suddenly drops down to subnormal before death ensues, but when death occurs early, the temperature usually remains high, whilst in a few instances it has been found to fluctuate within wide limits. A careful examination of blood smears taken at the first rise of body temperature frequently reveals the presence, although in very rare numbers, of either the parasites or Koch's bodies, and in a few instances both have been observed in the same smear, but the presence of these is demonstrable in large numbers only from about the third day of the disease.

In fatal cases, the disease usually runs a course of 3 (Fig. 2) to 11 days, the average of about thirty-five cases being 5·5 days. Recovery, when it takes place, extends over a period of 4 to 17 days, the average of nine recovered cases being 8·2 days (Fig. 3). It is of interest that in a few instances the parasites continued to make their appearance, although in very small numbers, in the blood circulation for about 2 days after the animals had, to all appearances, accomplished a clinical recovery and had resumed their normal feeding.

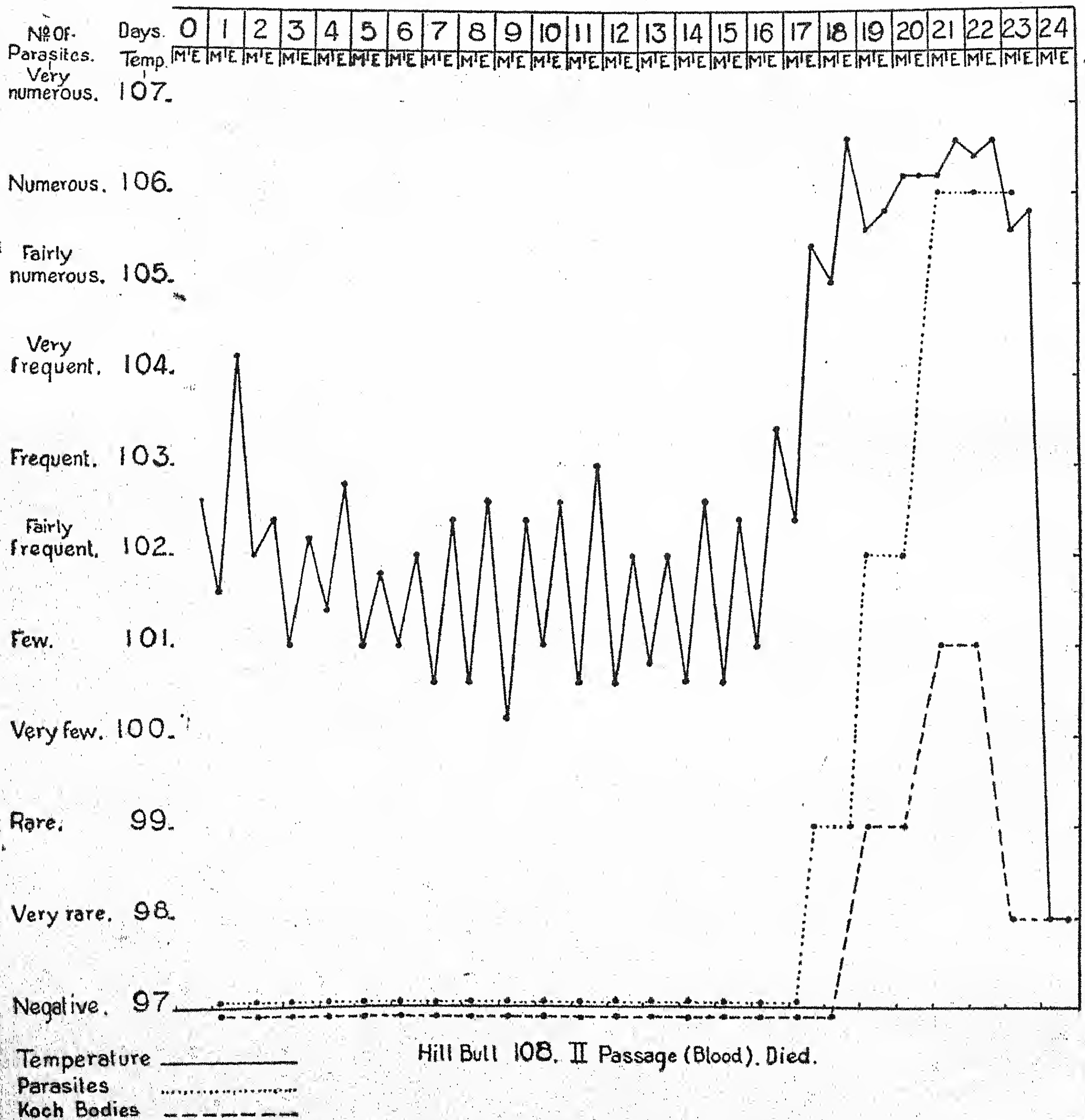


FIG. 1. Somewhat prolonged incubation period (17 days). Temperature drops suddenly to subnormal before death occurs.

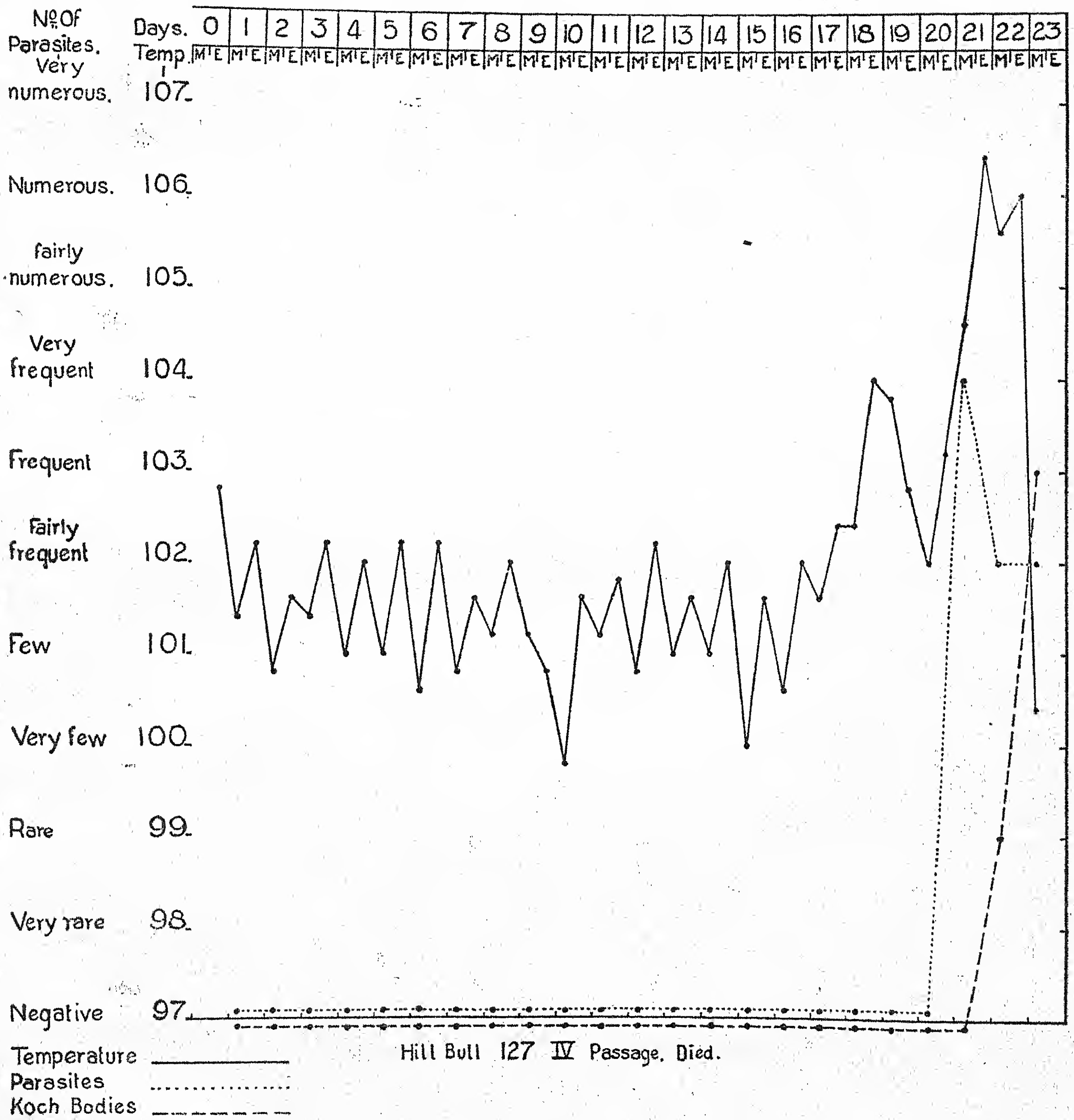


FIG. 2. Incubation period very much prolonged (21 days). Duration of illness very short (about 3 days).

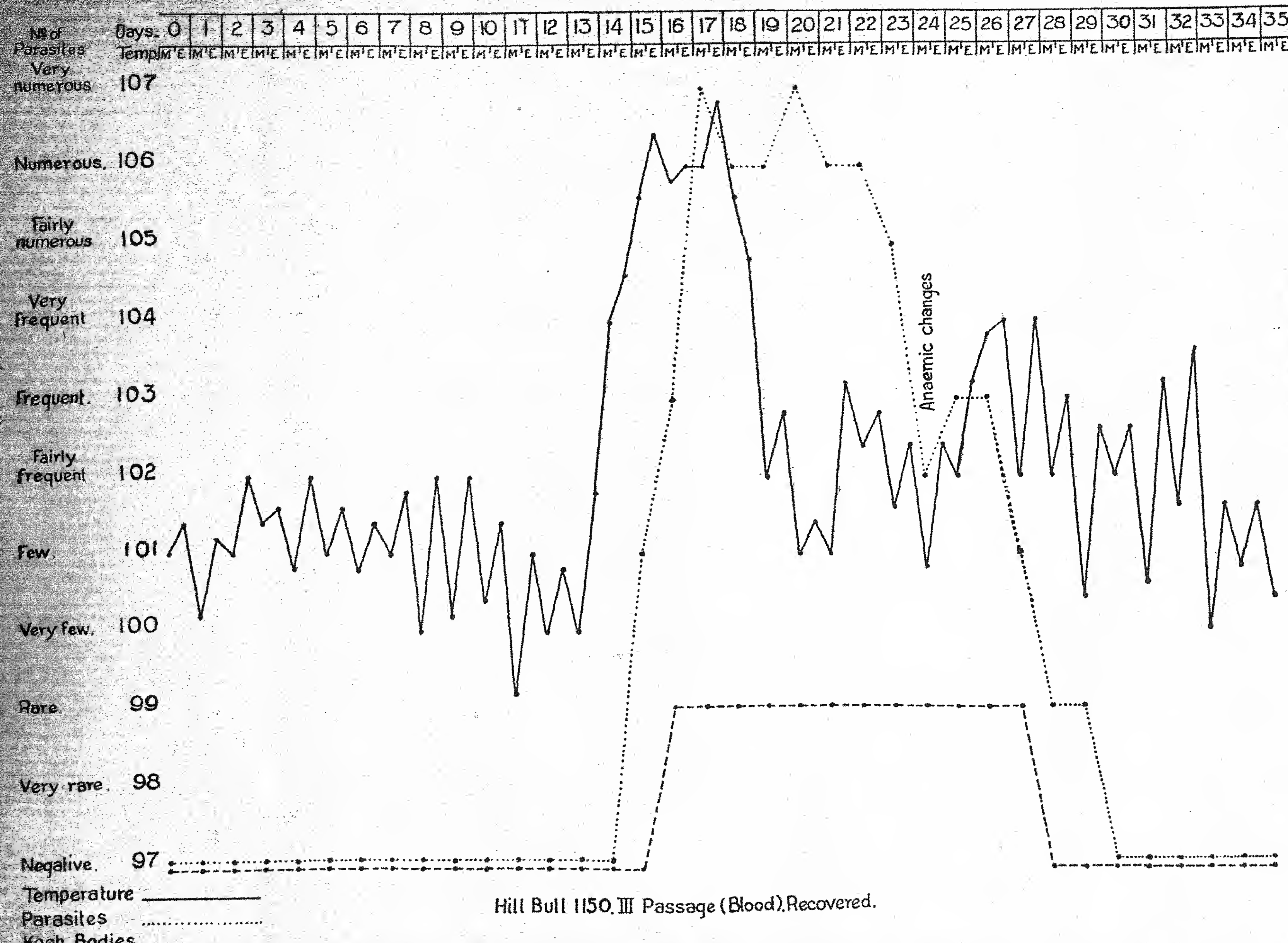


Fig. 3. Shows nature of reaction in a recovered case. Anaemic changes, indicating favourable prognosis, commenced immediately after the period of crisis.

The symptoms described above present a striking contrast, in several essential points, to those observed at Lahore, during November 1928, in an outbreak of natural theileriasis which occurred amongst a batch of twelve pure-bred Friesian stud bulls imported from South Africa. These observations, as made by Dr. J. T. Edwards and incorporated in a manuscript report left by the late Mr. Hugh Cooper, were as follows: "The symptoms of the disease consist of suddenly occurring and prolonged fever. The temperature remains constantly very high (105° to 107°F.) but no other symptoms are exhibited for the first few days. As soon as parasites (*T. mutans*) appear in the blood stream, however, the temperature begins to waver, to fall and rise again, but usually it never becomes so high as in the early stage. It is with the appearance of the parasites in the blood that other symptoms are shown. The most striking and characteristic symptom is profuse lachrymation. A watery discharge may also exude from the nostrils. The affected animals exhibit pica, or depraved appetite, to a marked degree. The coat becomes harsh and 'staring' and the animals become progressively weaker, and lose condition, when the superficial lymphatic glands are seen distinctly protruding beneath the skin. The loss in condition occurs in spite of the fact that the animals may feed quite well until within a day or two of death. The flanks particularly become hollow, and in the bull which died at Lahore breathing became hurried and apparently very painful, as seen in pleurisy. This symptom might have been attributable to the extreme anaemia exhibited. Estimations made show that the red blood-corpuscles are probably reduced in some cases to as low as about one-fifth of the normal. The visible mucous membranes become markedly anaemic in appearance, and in the latter stages some degree of icterus is present. Diarrhoea may supervene, and death is usually protracted. In the bull which died at Lahore, until very shortly before death it appeared that recovery might still take place, as parasites had become markedly reduced in number in the blood, and the animal was still able to move about readily". It should be mentioned that the rate of mortality in this outbreak was relatively low, for death occurred only in three (25 per cent) out of the twelve animals affected, the degree of severity of the affection observed in the remaining nine animals being stated to be as follows :—"Very severe affection, 1 ; marked affection, 6 ; marked affection with relapse, 2." It is noteworthy that the appearance of the parasites at a late stage of paroxysm (on the seventh day) also constituted a feature of the disease which occurred amongst a batch of imported pedigree bulls of American origin, at Allahabad, early in 1927 [Edwards, 1930].

Symptoms of lachrymation, associated with catarrhal rhinitis and an enlargement of the superficial glands, were also a characteristic feature of another outbreak of theileriasis which occurred, during 1930, amongst a batch of imported Dutch bulls, at the Military Dairy Farm, Kirkee (Poona), whilst some of these are also reported to have developed "pica" during the course of their voyage to India.

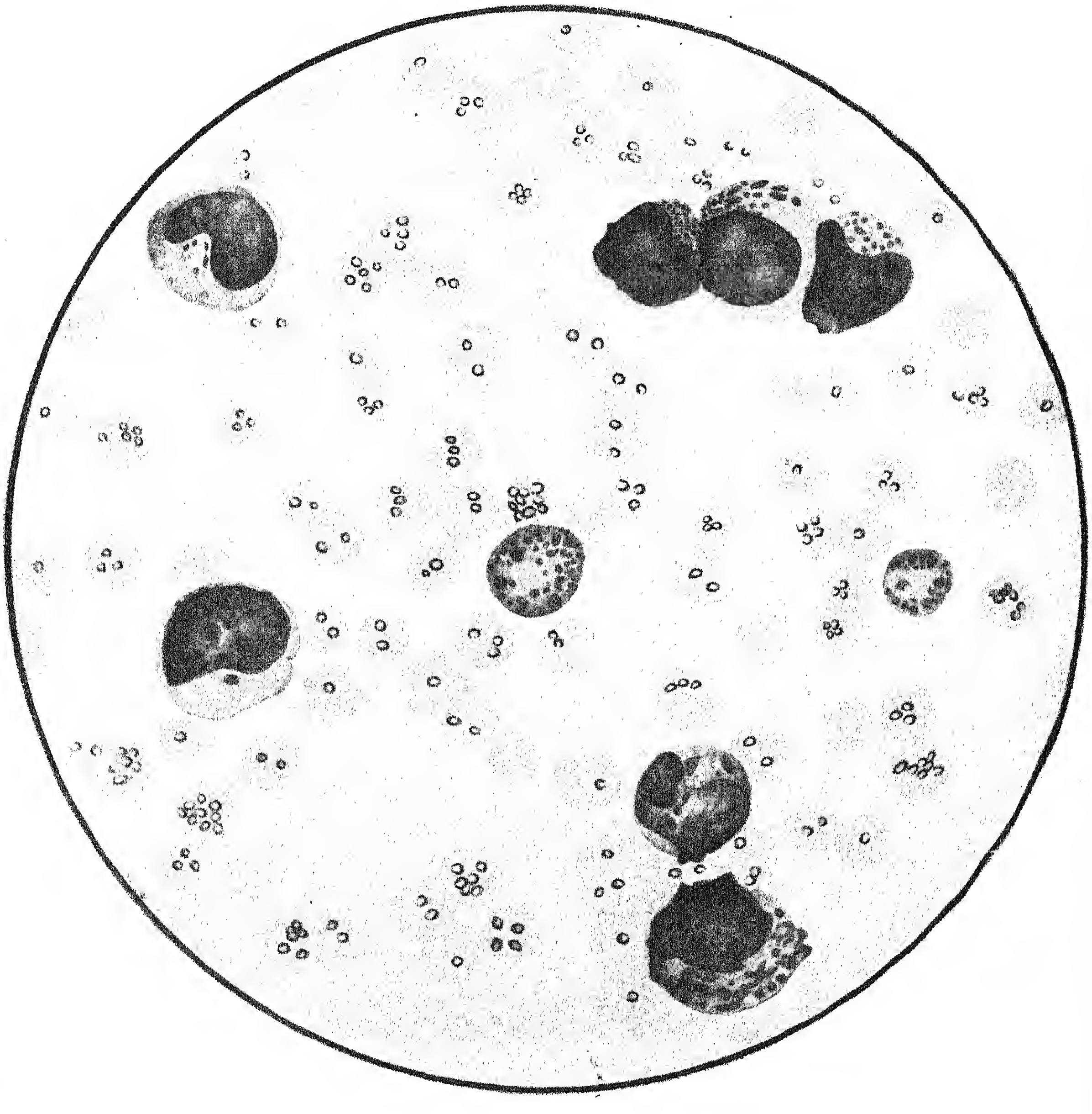
On the other hand, the thermal symptoms observed in these animals were usually of a remittent type, although in a few instances the temperature continued to remain high and even went up to 107°F. The condition in the Kirkee outbreak was, to all appearances, a chronic form of theileriasis, as is evidenced by the history of some of the animals. Thus, in one instance, the disease, after running an indifferent course, with periods of prolonged absence of any clinical symptom, suddenly assumed an acute form and progressed to a fatal issue after lasting for only one or two days. The examination of blood smears from the affected animals, however, showed, as a rule, the presence of only rare Theileria parasites and Koch's bodies, but in one instance the blood smears showed numerous parasites in the acute stage of the disease, about 50 per cent of the red blood-corpuscles being invaded, although the anaemic changes were almost negligible. It is worthy of note that in the Kirkee outbreak death occurred in only three out of a batch of ten affected bulls (30 per cent), so that this relatively low mortality rate presents a striking parallel to what was found to be the case in the Lahore outbreak.

Blood picture

As already mentioned, the parasites make their appearance within the first few (usually one to three) days of the first rise of body temperature, although at this time they occur in extremely small numbers, not more than one parasite being seen in any one corpuscle. As the disease, however, advances, they rapidly increase in number and may eventually invade 50 to 100 per cent of the red blood-corpuscles, as many as ten parasites having been seen in a single corpuscle. In regard to Koch's bodies, these usually vary in number from "rare" to "numerous", a maximum of ten having been seen in a single field. Both forms (gamonts and agamonts) have been observed and it is also not unusual to encounter occasional examples of "split up" forms. Koch's bodies have also been encountered in preparations made from the internal organs, *e.g.*, lymphatic glands, spleen, liver, lungs, kidneys, testicles and even from lesions in the stomach.

The Theileria parasites are usually seen as round or slightly oval forms, having the appearance of signet rings (Plate VII), and they are mostly uniform in size, measuring from one to two microns in diameter. The chromatin, particularly that at the peripheral portion of the parasite, takes the stain well, two chromatin spots being sometimes connected together, but the cytoplasm, which takes a pale blue colour when stained with Giemsa's or Leishman's stain, can only be seen in outline. It is extremely rare to encounter the rod forms of the parasite, and when they are present they presumably represent the "carrier" forms of *T. mutans*, inasmuch as the latter are commonly met with even in the incubative stage of the disease.

It is of interest that *Babesia bigemina*, in "rare" to "very large" numbers, was observable in more than 60 per cent of the bulls that reacted to artificial infection with theileriasis, both during the period of incubation and in the early



Theileria sp. (Type B), associated with Koch's blue bodies, as seen in acute infections in Indian (Hill) cattle × 1,000.

Post-mortem appearances

The most constant post-mortem findings are a thin and watery character of the blood, oedema and enlargement of the superficial and internal lymphatic glands and of the spleen and a gelatinized condition of the body fat. There are petechiae of the serous and mucous membranes and also of the epicardium and endocardium and large numbers of irregular petechial marks on the larynx, pharynx and the trachea. The lungs are frequently oedematous, whilst the abomasum usually shows characteristic ulcers which may vary in size from a pea to a rupee coin, these ulcers consisting of a central block of necrotic area surrounded by a haemorrhagic zone. Similar ulcers, associated with petechial marks, may be found along the whole length of the intestines. There may be haemorrhagic infarcts present in the kidneys, but this has not been found diagnostic of the condition. The thoracic and abdominal cavities usually show varying quantities of serous and sero-sanguineous fluid.

Unfortunately little exact information, for comparison with the foregoing description, is available concerning the post-mortem appearances in the cases of natural theileriasis that have occurred amongst imported Friesian bulls in this country. In one of the animals that died at Lahore, the liver showed necrotic patches with a yellow centre and a deep greenish periphery and extending throughout the parenchyma, whilst the bile was "thick like the consistence of thin porridge". The abomasum showed many minute petechiae confined only to the centre, whilst the mucous membrane was found to contain "intense lesions of a peculiar kind consisting of innumerable circumscribed small patches in all stages of development into wellformed ulcers". The post-mortem appearances in the case of two imported bulls which died at Kirkee consisted of an enlargement of the liver and spleen, and in one of the animals the kidney, too, was enlarged, whilst in the other there was evidence of vegetative epicarditis, associated with the occurrence of petechiae under the epicardium.

Viability of strain

A short series of experiments was carried out to determine the length of time for which Theileria parasites remain viable both at room temperature (21°-22° C.) and in the refrigerator. For this purpose, blood showing large numbers of these parasites was drawn under sterile precautions and defibrinated before storage and its infectivity was tested, at different intervals, upon bulls. It was observed that blood kept at room temperature remained infective up to 96 hours and that stored in the refrigerator completely lost its infectivity within six days.

Localization of "virus"

In view of the opinion expressed by Richardson [1930] that the causal agent of East Coast Fever might in reality be an ultra-visible virus, a series of experiments, as detailed below, was carried out to test the infectivity of serum and laked blood after these had been filtered.

Experiments with filtered serum.—Blood was drawn separately from two acute cases of artificial theileriasis showing large numbers of Theileria and Koch's bodies and was allowed to clot. The serum after separation was filtered through a Seitz bacterial filter and each filtrate was injected into two bulls in about 10 c. c. doses, the interval between the drawing of blood and the injection of the filtrate being about 26 hours. The results were, however, entirely negative, and when later tested for immunity by the injection of virulent blood, all the four animals proved susceptible to the disease.

Experiments with filtered blood.—Blood from an acute case of artificial theileriasis was defibrinated and haemolyzed by shaking with an equal quantity of sterile distilled water. It was then filtered through a Seitz bacterial filter and the filtrate injected intravenously, in quantities of 20 c.c. each, into two bulls, but the results were negative. Both the bulls proved susceptible to theileriasis when later tested for immunity with virulent blood.

Immunity tests

The results of immunity tests are summarized in Table II.

Table II

Showing the results of Immunity test on recovered cases

Serial No.	Hill Bull No.	Date of infection	Interval between recovery and test	Result	Remarks
1	537	30th July 1931 .	About 78 weeks .	Immune	Natural case treated with Plasmoquine.
2	14	20th June 1932 .	" 35 " .	"	Experimentally infected and naturally cured.
3	1150	28th July 1932 .	" 29 " .	"	Do.
4	101	27th September 1932	" 22 " .	"	Experimentally infected and treated with Todorit.
5	246	24th September 1932	" 22 " .	"	Experimentally infected and treated with Piro-blue.
6	167	24th September 1932	" 22 " .	"	Experimentally infected and treated with Todorit.
7	296	29th October 1932 .	" 18 " .	"	Experimentally infected and treated with Trypaflavine.
8	119	19th December 1932	" 12 " .	"	Experimentally infected. Reaction mild. Recovery natural.
9	488	19th December 1932	" 12 " .	"	Experimentally infected and treated with Plasmoquine.
10	761	5th February 1933 .	" 5 " .	"	Experimentally infected and treated with Sulfarsenol.

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An examination of this table shows that animals after recovery from the infection develop what, to all appearances, is a solid and sterile immunity, this being in contrast with the type of immunity observed in the imported bulls, for in the latter case "it is believed that recovery from an attack leaves no real immunity and that the animals almost certainly remain ' carriers ' of the parasite and are liable to relapses at any subsequent time " [Cooper]. It will be observed that in three out of the ten animals subjected to these tests, recovery had occurred spontaneously, and this may afford an explanation for the fact that, as already mentioned, nearly 47 per cent of the animals sub-inoculated with blood derived from acute cases of theileriasis failed to develop the infection, for the resistance exhibited by these animals might have been due to their having had attacks of the disease early in life. A possibility of this kind is suggested by the fact that, according to Walker [1930], " in young calves infested by hand with ticks containing a pure infection of *Theileria parva* it was not unusual to find a lower percentage mortality as compared with adults infested by hand with the same batch of infected ticks ". On the other hand, in a recent communication MacHattie [1935], in Baghdad, has recorded the occurrence of a fatal outbreak of *T. annulata* infection amongst a batch of dairy calves which were only two to three weeks old, whilst sporadic instances of similar infection have also been mentioned by Mason [1922], Sergent and his collaborators [1927], Turnbull [1926], and Theiler and Graf [1928].

It is worthy of note that, with a few exceptions, all the animals in this investigation had been previously utilized in rinderpest work, and whether this fact in any way determined the development of the theileriasis reaction in them is a matter that would seem to demand attention.

IDENTITY OF PARASITE

In the confused state of our present knowledge concerning the systematic position of the theileria parasites in general, it would be hardly profitable to enter upon a lengthy discussion of the specific identity of those involved in acute or fatal cases of the disease in India. As already indicated in the introduction, the problem of theileriasis itself requires further clarification before any useful discussion can be undertaken concerning the identity of the parasites. Thus, the condition met with in India would appear to comprise (besides *T. mutans* infection) two distinct forms, which, for the sake of convenience, may be designated as Type A and Type B, their position in du Toit's [1930*a*] scheme of classification being as follows :—

TABLE III

Showing the difference between various strains of Theileria including those occurring in India

(After du Toit)

Serial No.	—	*T. parva*	*T. dispar*	*T. annulata*	*T. mutans*	*Theileria* sp. (Type A)	*Theileria* sp. (Type B)
I	Endoglobular form	Over 80 per cent rod shaped. Less than 20 per cent round or oval.	Less than 10 per cent rod shaped. More than 90 per cent round or oval.	About 20-30 per cent rod shaped. 70-80 per cent round or oval.	About 45 per cent rod shaped. 55 per cent round or oval.	A very large proportion (exact percentage not worked out) round.	90-100 per cent round.
II	Koch's bodies	Always present usually very numerous.	Always present, in severe cases usually easily demonstrated.	Only present in acute cases but then very numerous also in the blood.	Extremely rare, in most cases cannot be found at all.	As a rule rare, but have been found in very large numbers in a few cases.	Always present in blood.
III	Persistence of parasites.	Disappear completly after recovery.	Disappear completely after recovery.	Persist in blood throughout life.	Persist in blood throughout ife.	Persist in blood throughout life.	Disappear completely from blood after recovery.
IV	Transmission with blood.	Possible but very difficult.	Easy even with small quantities.	Easy even with small quantities.	Easy even with small quantities.	Not known.	Easy even with small quantities.
V	Natural Transmission.	Through *Rhipicephalus* spp.	Through *Hyalomma mauretanicum.*	Not known.	Through *Rhipicephalus* spp.	,,	Not known.
VI	Average incubation period.	About 13 days.	About 21 days.	More than 20 days.	About 30 days.	,,	Average of about 145 cases 16 days.
VII	Type of infection.	Always acute.	Always acute.	Chronic but may become acute.	Always chronic.	Chronic but may become acute.	Acute.
VIII	Anaemia.	Absent or only slight Anisocytosis.	Present. Anisocytosis. Polychromatophilia.	Present in acute cases.	Absent or slight.	Present in acute cases.	Present when recovery takes place.
IX	Icterus	Very rarely present.	Frequently present.	Sometimes present.	Absent.	Sometimes present.	Often present.
X	Haematuria.	Absent.	Sometimes present.	Absent.	Absent.	Absent.	Absent.
XI	Mortality.	90-100 per cent. In enzootic regions less.	About 20 per cent.	5-20 per cent.	*Nil.*	25 to 30 per cent.	Over 75 per cent.
XII	Lymph glands.	Always swollen.	Swollen.	Often swollen.	Normal.	Swollen.	Always swollen.
XIII	Spleen.	Normal or only slightly swollen.	Very much swollen.	Very much swollen in acute cases.	Normal.	Swollen.	Always swollen, oedematous and congested.
XIV	Kidneys.	'Infarcts' present	'Infarcts' present.	Small haemorrhages present.	Normal.	No special character mentioned.	Both 'infarcts' and haemorrhagic spots present.
XV	Liver.	Only microscopic changes.	Swollen.	Swollen.	Normal.	Swollen.	Very much swollen.
XVI	Abomasum and Intestines.	Ulcerations present.	Ulcerations present.	Ulcerations present.	Normal.	Ulcerations present	Ulcerations present.
XVII	Haemorrhages	Absent.	Present in heart and other organs.	Often present.	Absent.	Not mentioned.	Absent.
XVIII	Immunity	Solid and sterile.	Solid and sterile.	No immunity but state of premunition.	No immunity but state of premunition.	No immunity but state of premunition.	Solid and sterile.

It will be seen from the foregoing table that while Type A possesses characters which are mostly identical with those described for *T. annulata*, Type B, although it has the largest number of diagnostic points in common with *T. dispar*, nevertheless differs from it in the considerably higher rate of mortality.

It is to be mentioned, however, that du Toit [1930*b*] has expressed the opinion that the name *dispar* should be regarded as a synonym of *annulata* and that the latter should be regarded as valid on grounds of priority, so that there are only three species of Theileria of cattle, namely, *T. parva*, *T. annulata* and *T. mutans*.* This view would also appear to be largely shared by Theiler [1930] himself, as is evidenced by the expression "*T. dispar* or *annulata*" frequently used by him. On the other hand, Donatien and Lestoquard [1931] have contended that it is *T. annulata* that should be sunk as a synonym of *T. dispar* and *T. mutans*, whilst Sergent, Donatien Parrot and Lestoquard [1932] have even referred to the possibility of *T. dispar* undergoing a morphological modification towards the end of the acute stage of the disease and becoming completely attenuated in virulence (presumably to the extent of being indistinguishable from *T. annulata*).

It seems to the present writers that the case for *T. annulata* is better than that for *T. dispar*, for whereas the latter name is almost exclusively applied to the parasite found in Algeria, *T. annulata* (or parasites almost identical with it) has been described from several countries around the Mediterranean Sea, including those bordering on Algeria itself, *e.g.*, Tunis [Ducloux, 1905] and Morocco [Velu and Eyraud, 1915], so that it would appear more than probable that the Algerian infection is merely a variant of the more widely distributed form of theileriasis which goes by the name of Mediterranean Coast Fever and is believed to be due to *T. annulata*.

It has, however, been pointed out recently by Yakimoff and his collaborators [1932], that the proportion of round and rod forms of these parasites varies within such wide limits in the different forms of theileria infections that this character can hardly be of any value for classificatory purposes, so that, considering the acuteness of the infection, the rate of mortality and the type of immunity reaction, it cannot be regarded as altogether improbable that Type B represents merely a variant of *T. parva*, but of depressed virulence.

On the other hand, the evidence is so overwhelming in favour of the view that the parasite (Type A) concerned in outbreaks of acute theileriasis amongst imported Friesian bulls belongs to the species *T. annulata*, that it is only necessary to consider the possible manner in which these animals might have contracted the infection. An examination of the history of some of these animals shows that, prior to embarkation from South Africa, they were immunized against African "piro-

* This is why Type B has been provisionally designated as *T. annulata* in the Annual Report of this Institute for the year ending March 1933 (p. 29).

plasmosis ", by which term *B. bigemina* infection is doubtless meant. It is therefore reasonable to conclude that the clinical symptoms exhibited by a proportion of these animals while *en route* to India, as already mentioned earlier in this paper, cannot have been those of redwater, nor of *T. parva* infection, for in the latter event, the disease would not have run a chronic course, as was found to be the case with a large majority of the animals. The possibility, therefore, suggests itself that the organisms were acquired during the course of the voyage, the infecting material having been, in all probability, tick-infested fodder. Evidence of this is further afforded by the fact that the records in the possession of this Institute contain particulars of two cases of theileriasis which occurred among Friesian bulls imported direct from Holland. It would be idle to dogmatize as to the exact locality where the infection might have been contracted, but it is probable that this occurred at some point along the Mediterranean Coast, where, as is well known, *T. annulata* infection is widely prevalent.

Curative treatment and prophylaxis

A fairly large number of drugs—mostly chosen on account of their known parasiticidal value—were tested as to their efficacy for this condition, the dosage employed having been in accordance with the schedule laid down by the manufacturers themselves or based upon experience previously obtained in the use of these drugs in the treatment of other diseases. As the results of these trials were, for the most part, highly unsatisfactory, it would be hardly profitable to consider them in detail. They are, however, summarized in the following table :—

Table IV

Serial No.	Drug	Number of animals treated	Number of animals cured
1	Piroblue	5	1 (or 20·0 per cent)
2	Todorit	6	2 (or 33·3 per cent)
3	Trypaflavine	5	2 (or 40·0 per cent)
4	Antimosan	11	*Nil.*
5	Plasmoquine	8	1 (or 12·5 per cent)
6	S. U. P. 36	3	*Nil.*
7	Sulfarsenol	9	1 (or 11·1 per cent)
8	Novarsenobillon	6	2 (or 33·3 per cent)
9	Acriflavine	11	3 (or 27·3 per cent)
10	Myosalvarsan	3	*Nil.*
11	Mercuric chloride	6	2 (or 33·3 per cent)
12	Bayer 205	7	3 (or 43·0 per cent)
13	Antimony tartrate	5	1 (or 20·0 per cent)
14	Gonacrine	7	3 (or 43·0 per cent)
15	Sodium cacodylate	6	1 (or 16·7 per cent)
16	Asaphelene	5	1 (or 20·0 per cent)
17	Bayer 205 and Tartar emetic	11	2 (or 18·1 per cent)
18	Quinine hydrobromide	5	1 (or 20·0 per cent)
19	Atebrin and Plasmoquine	8	1 (or 12·5 per cent)
20	Atebrin	19	11 (or 57·8 per cent)
	Total	146	38 (or 26·0 per cent)

It will be seen that recovery was obtained in only 38 of the 146 animals (26 per cent) used in these trials and that the best results were obtained with Atebrin, whilst "Bayer 205" and Gonacrine ranked second in the list. On the other hand, some allowance has to be made for the fact that, in a small proportion of cases, recovery has been found to occur spontaneously, so that the actual efficacy of these remedies is probably less than that indicated by the percentages worked out above.

A short series of experiments was also carried out to test the value of the so-called "premunition" against this disease, in view of the highly encouraging results reported to have been obtained by Sergent and his collaborators [1931] against *T. dispar* infection in North Africa by the adoption of this method. Twelve bulls were each injected subcutaneously with 20 c. c. of blood drawn at the initial stage of the disease, *i.e.*, on the very first appearance of the febrile symptoms, it being assumed that the blood so drawn would be free from parasites or Koch's bodies—an assumption which also received support from the fact that the results of examination of blood smears taken at this stage of infection were consistently negative. Three of the twelve bulls mentioned above showed a definite reaction, as a result of the injection, after the usual incubation period and one of them eventually died after showing symptoms of acute theileriasis. Six animals showed a mild reaction lasting only for a day or two, whilst the remaining three animals did not develop any symptoms at all. All the eleven surviving animals proved resistant to theileriasis when tested by the inoculation of virulent blood about six weeks later.

Summary and conclusions

The acute or fatal forms of bovine theileriasis in India resolve themselves into two well-differentiated categories, as described below:—

(1) *Theileriasis in imported Friesian bulls.*—The disease usually runs a chronic course with periods of prolonged absence of any clinical symptoms, but it may suddenly assume an acute form and rapidly progress to a fatal issue. The affected animals exhibit an elevation of body temperature and sometimes also symptoms of "pica", whilst the visible mucous membranes are often markedly anaemic, although this last may sometimes be negligible. Lachrymation and marked swelling of the superficial lymphatic glands are also fairly constant features of the disease. The examination of blood smears frequently shows only rare or few parasites and Koch's bodies, but in a proportion of cases they may occur in large numbers when the disease is at its height, the majority of the parasites being round or oval in form and nearly 50 to 70 per cent of the red blood-corpuscles being invaded by them. There is no solid or sterile immunity in this condition, but a state of premunition, and recovered animals are liable to relapse. The rate of mortality amounts to 25 to 30 per cent. The outstanding features of post-mortem appearances are an enlargement of the liver and spleen and the occurrence of petechiae in various organs,

The species of parasite concerned in this form of theileriasis possesses characters which are mostly identical with those described by du Toit [1930 *a*] for *Theileria annulata*, and all available evidence points to the conclusion that the infection is exotic in origin, the organisms being probably acquired by the animals during the course of their voyage to India.

(2) *Theileriasis in Indian cattle.*—This condition, which forms the main subject of the present paper, has been extensively studied in artificially infected hill bulls, the disease having been successfully reproduced in them by the inoculation of virulent blood and organ emulsions, although nearly 47 per cent of the animals proved refractory to the infection.

The average incubation period in artificial infection is 16 days, and in fatal cases the average duration of the disease is 5·5 days, but when recovery occurs, it extends over a period of 4 to 17 days. The principal symptoms consist of an elevation of the body temperature, progressive inappetence and an enlargement of the prescapular and precrural glands, whilst the visible mucous membrane of the eyes shows varying degrees of icterus and petechiae. In a number of cases, the temperature suddenly drops down to subnormal before death ensues, but when death occurs early, the temperature remains high. The examination of blood taken at the first rise of temperature frequently reveals the presence of rare parasites and Koch's bodies or both, but the parasites rapidly increase in number as the disease advances and may eventually invade 50 to 100 per cent of the red blood-corpuscles, although Koch's bodies may vary in number from "rare" to "numerous". The parasites are usually seen as round forms, the "rods" being rarely encountered. The rate of mortality amounts to more than 75 per cent.

The sequence of changes occurring in the blood of an affected animal has been found to furnish a fairly reliable indication as to its chances of recovery. In the event of the prognosis being good, the first noticeable change in the blood picture is the occurrence of anisocytosis, this being followed by polychromatophilia and granular basophilic degeneration.

The most constant post-mortem findings are a thin and watery character of the blood, oedema of the lymphatic glands and of the spleen and a gelatinized condition of the body fat. There are petechiae of the serous membranes and respiratory tract, whilst the abomasum may show characteristic ulcers.

The parasites remain viable up to 96 hours at 21°—22°C., and when stored in the refrigerator they lose their infectivity within six days. Blood showing large numbers of parasites and Koch's bodies was filtered and the infectivity of the filtrate was tested on bulls, but the result was negative. The serum derived from such blood and treated in the same manner likewise proved non-infective.

Twenty drugs were tested as to their efficacy for this condition, but the results were unsatisfactory. A short series of trials was also carried out to test the value of premunition against the disease and the results were encouraging.

The species of parasite concerned in this form of infection presents a close resemblance to *Theileria dispar*, as described by du Toit [1930*a*].

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*PNEUMONIA IN FOALS DUE TO *CORYNEBACTERIUM EQUI*

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(With Plates VIII—XI and two text-figs.)

INTRODUCTION

AMONG the various diseases which afflict young foals in the breeding establishments of India, strangles, joint-ill or pyo-septicaemia due to streptococcus, *S. abortus equi*, *Shigella equirulis*, and *Escherichia coli*, and pneumonia due to streptococci are well known. An examination of the history of foaling of any of the large breeding establishments will, however, usually reveal a number of other losses variously ascribed to causes such as high-fever, pneumonia, suppressed strangles, colic, diarrhoea, bronchitis, pyaemia and debility, and it will be shown in this article that a large number of such cases, especially those denoted as pneumonia occurring within the first 12 weeks or so of birth, are due to a specific micro-organism, a pigmented diphtheroid, namely *Corynebacterium equi*. It is also probable that a large percentage of cases known as suppressed strangles are due to infection with the same micro-organism, as the first few cases from which material was submitted for examination, and from which knowldege was first obtained with regard to the precise cause of the pneumonia, were forwarded to us as possible cases of suppressed strangles.

* Paper read before the Indian Science Congress, Indore, 1936.

A thorough search into the current periodical literature has shown that an organism identical with the one found in India has been previously incriminated in certain other countries as a cause of pneumonia in foals, although this has not yet been described in any text-books on veterinary medicine or bacteriology. This is, however, the first time that the occurrence of this organism as the specific cause of pneumonia in foals has been recorded in India, or indeed in Asia.

Magnusson [1923] was the first to describe a pigmented diphtheroid in association with specific pneumonia in foals and he recorded 12 cases of its incidence in a breeding establishment in Southern Sweden. To this organism he assigned the name *Corynebacterium equi*. Subsequently Meissner and Wetzel [1923], Lutje [1923] and Lund [1924] recorded its incidence in Germany. Outside Europe this condition has been described in South Australia by Bull [1924] and by Dimock and Edwards [1931] in America.

Since 1924, when this condition was first brought to the notice of this Institute, a number of cases have been known to occur year after year in at least two of the horse-breeding establishments in the Punjab. At first it was thought that the condition was a form of strangles, for, among a batch of foals in a paddock, several became ill, and some cases developed the typical sub-maxillary lesion of true strangles, while others developed into cases of pneumonia. Subsequent epidemiological observations and transmission experiments proved that the two conditions were quite distinct. Thus, pneumonic cases were known to occur during several seasons in which no strangles cases occurred: Out of a batch of 15 mares with foals at foot which had been made up straight from the foaling boxes (*i.e.*, the mares which foaled in the open were brought into a loose box for about 9 days and then drafted into a paddock) into a fresh paddock in which there had been no animals for several months previously and which had had no history of strangles, eight foals went down with fever and pneumonia, none of them showing external signs of strangles. Strangles was finally eliminated by reproducing the typical pneumonia in young foals with *Corynebacterium equi*, which had been maintained in subculture.

Although the disease may occur through all the seasons of the year, it will be observed from Fig. 1 that its incidence is highest in the summer months.

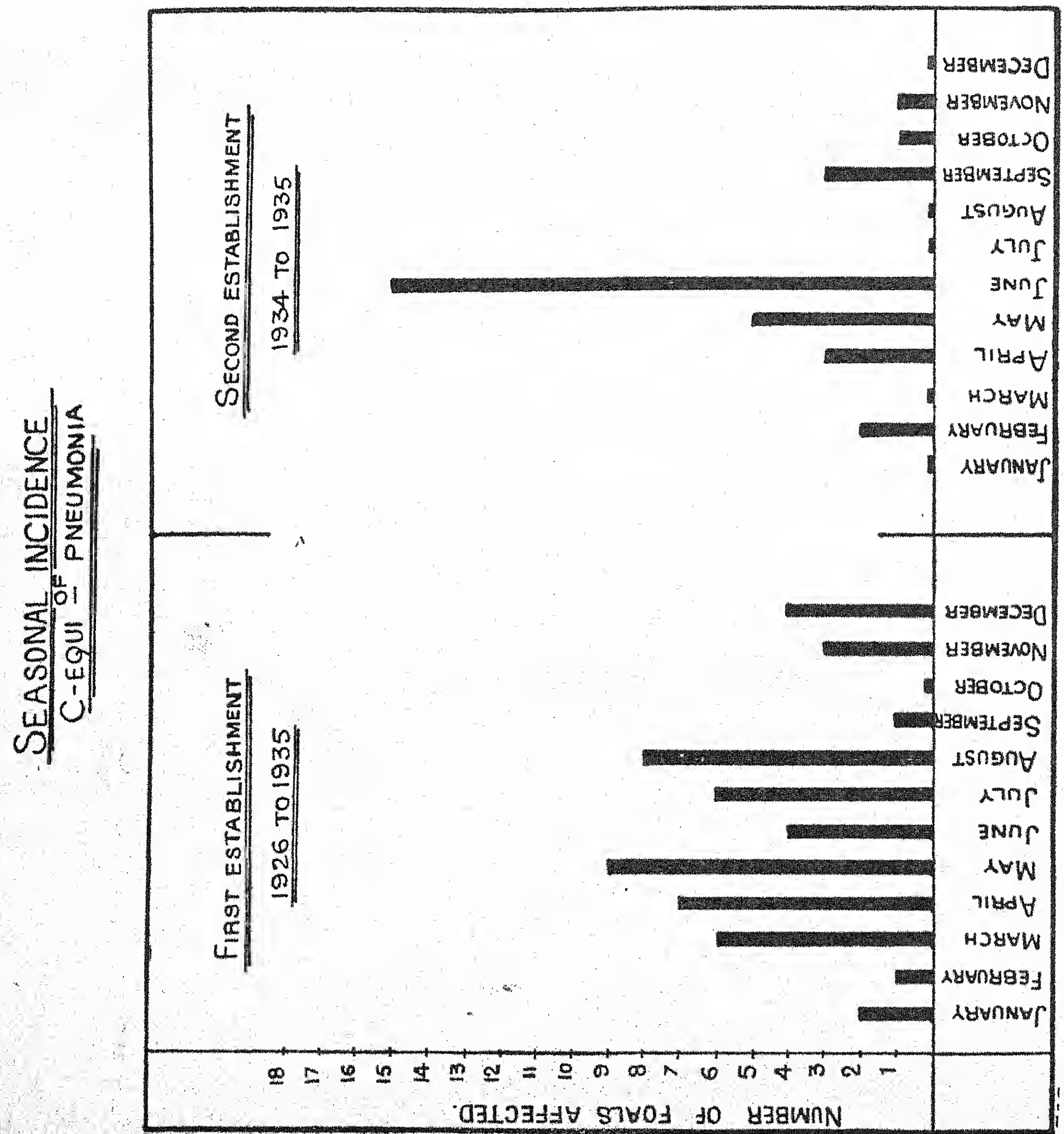

SEASONAL INCIDENCE OF C-EQUI PNEUMONIA
FIRST ESTABLISHMENT
1926 TO 1935
SECOND ESTABLISHMENT
1934 TO 1935
NUMBER OF FOALS AFFECTED.
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
JANUARY
FEBRUARY
MARCH
APRIL
MAY
JUNE
JULY
AUGUST
SEPTEMBER
OCTOBER
NOVEMBER
DECEMBER

FIG. 1.

FIG. 1. Foal No. 386. Infected by instillation of culture into the nostrils. Note the swelling of the hock joints.

FIG. 2. Natural case of *C. equi*—pneumonia in a foal with joint lesion, off-hock. (By courtesy of Major Sinton.).

There would also appear to be a definite age susceptibility to this disease. The writer had an opportunity to examine an extract of the history of foals bred in one of the infected breeding establishments, and here the average age at which death took place from pneumonia worked out at two months and six days. There were cases which died within one month of birth and rare cases which died at as late as 7 months, but the majority of cases (60 per cent) died between the first and third month of birth, *vide* Fig. 2.

Carne [1927] in Australia has described an atypical case of the disease in an eight-year-old mare. Here the chief lesion was located in the kidney in the form of a haemorrhagic nephritis, the lungs showing only a few pea-sized nodules in the place of the large abscesses that are found in the lungs in cases of the typical disease in foals.

Symptoms

The onset is marked by a low fever, which after a day or two may attain 104-105° F. or over. There is concurrently a watery discharge from the eyes and the nostrils. There is laryngitis with a sharp choking, dry cough. There is also snoring. The foal becomes very dull, its appetite is impaired and its pulse and respiration are increased. Occasional cases show symptoms of joint-ill (Plate VIII, figs. 1 & 2). Indeed, one of the cases that was forwarded to this Institute had actually been destroyed for joint-ill. The hock is the joint that is usually involved, the swelling sometimes extending to the fetlock. Soon, symptoms of pneumonia set in with marked distress in breathing, which is of the abdominal type. There is a double-heave in expiration. Moist rales and crackling gurgling noises may be heard on auscultation. There is constipation followed by diarrhoea with pale yellow frothy faeces. The foal loses weight, becomes very emaciated and dies within 8 to 10 days, although occasionally death may not occur for 20 days after the onset of the symptom. In foals that were artificially infected by the intra-nasal route, the period of incubation was found to be from six to eight days.

Post-mortem findings

The carcase is emaciated. The ribs are prominent, and where joint-ill is present, the hock-joint contains slightly haemorrhagic gelatinous fluid. On opening the thoracic cavity, the lungs are found to be studded particularly in the middle and the right lobes, with abscesses of the size of a small pea to that of an egg or even larger (Plate IX, fig. 1 & Plate X, fig. 1). These abscess cavities contain fluid or inspissated pus, which may emit a characteristically bad odour. The mediastinal lymph glands are enlarged and contain huge abscess cavities (Plate IX, fig. 2). The mucous membrane of the intestines is congested and contains slimy frothy

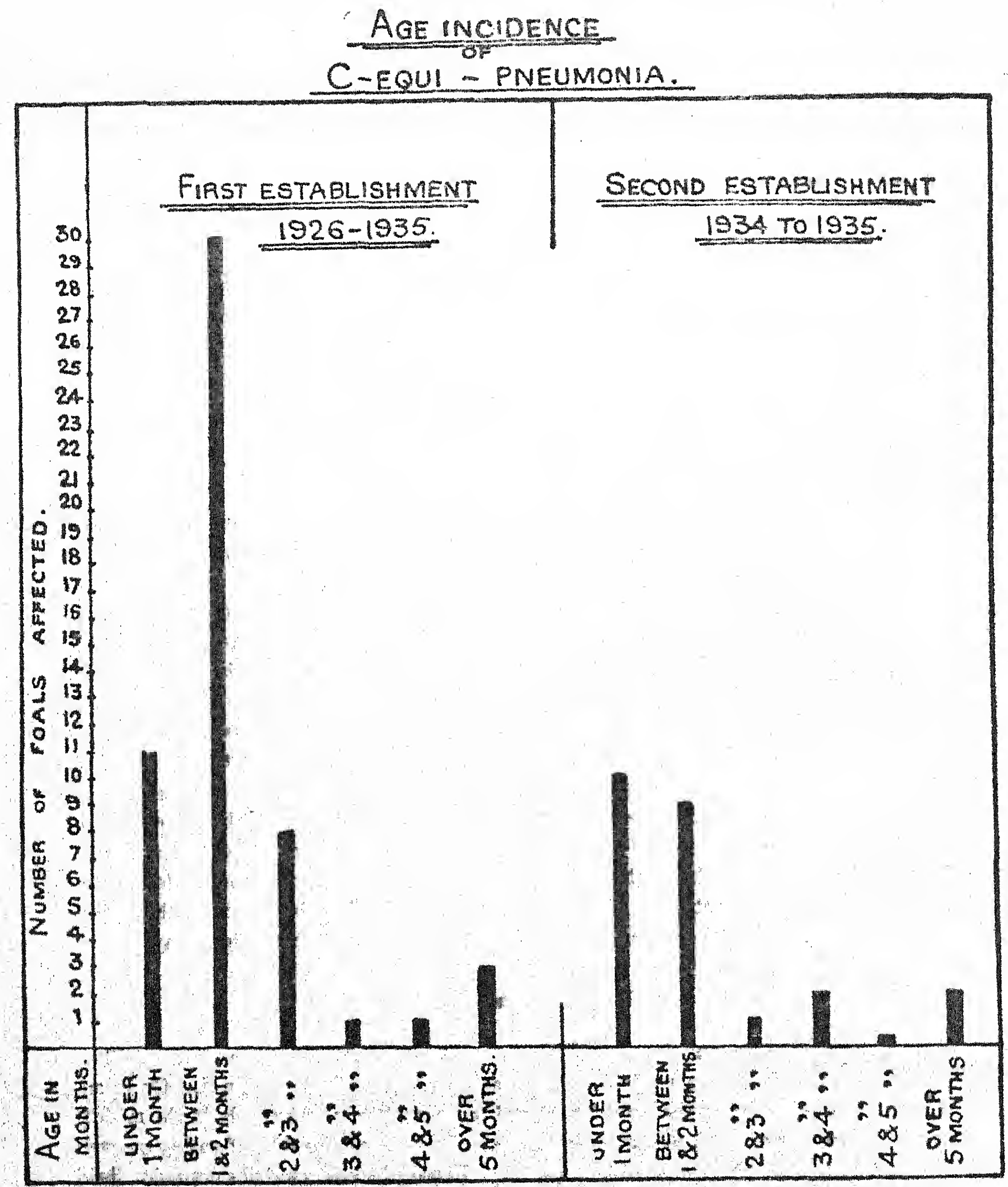

AGE INCIDENCE OF C-EQUI – PNEUMONIA.
FIRST ESTABLISHMENT 1926-1935.
SECOND ESTABLISHMENT 1934 TO 1935.
NUMBER OF FOALS AFFECTED.
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
AGE IN MONTHS.
UNDER 1 MONTH
BETWEEN 1 & 2 MONTHS
" 2 & 3 "
" 3 & 4 "
" 4 & 5 "
OVER 5 MONTHS.
UNDER 1 MONTH
BETWEEN 1 & 2 MONTHS
" 2 & 3 "
" 3 & 4 "
" 4 & 5 "
OVER 5 MONTHS

FIG. 2.

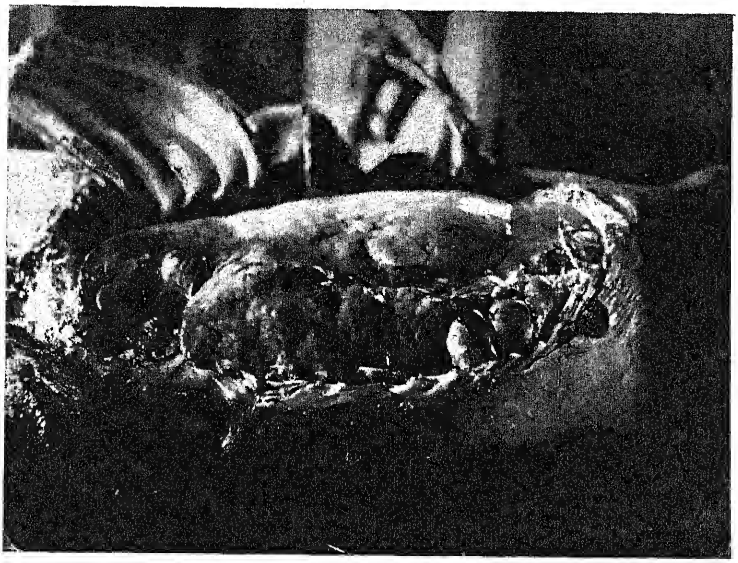


FIG. 1. Foal No. 386. The appearance of the lungs *in situ*. Note the bunch of abscesses and areas of hepatisation.

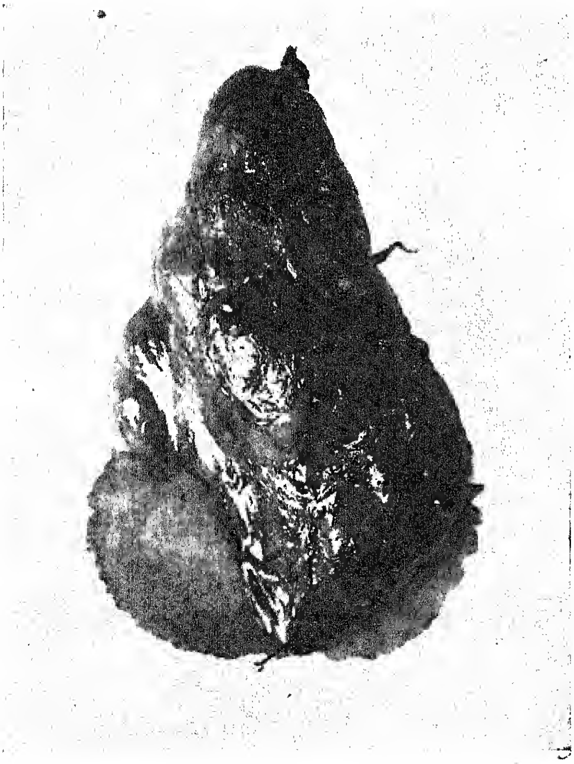


FIG. 2. Foal No. 386. The anterior mediastinal gland with abscesses.

FIG. 3. Microphotograph of *C. equi* Smear from 24 hours broth culture stained Leishman ($\times 1200$). Note the beaded appearance of the rods.

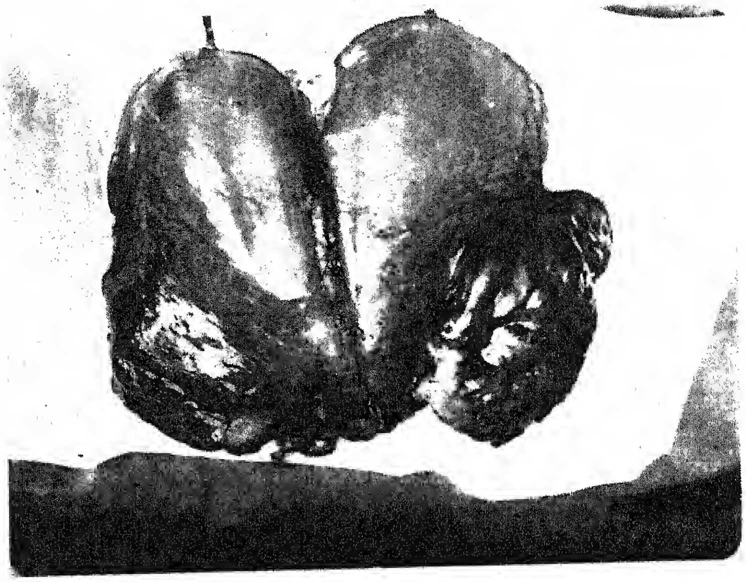


FIG. 1. Foal No. 386. The lungs.

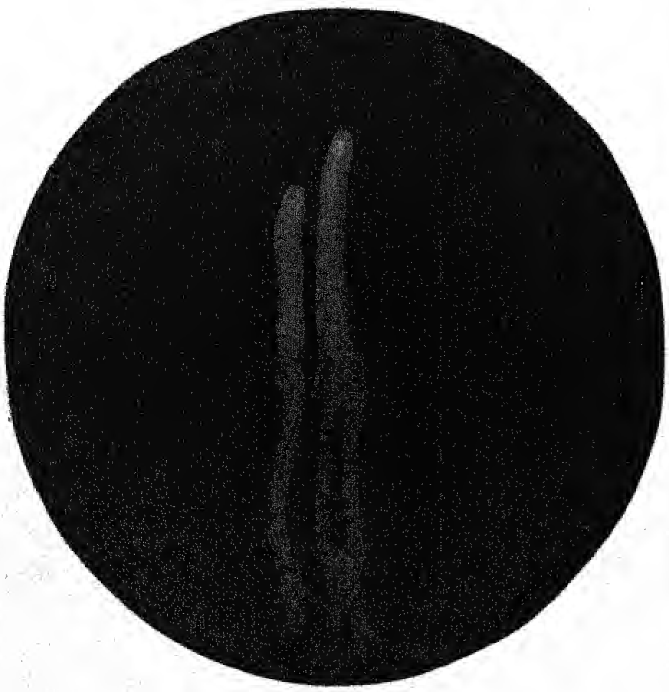


FIG. 2. *C. equi.* 36 hours' growth in a petri-dish kept in an upright position. Note the mucoid nature of the growth, flowing down.

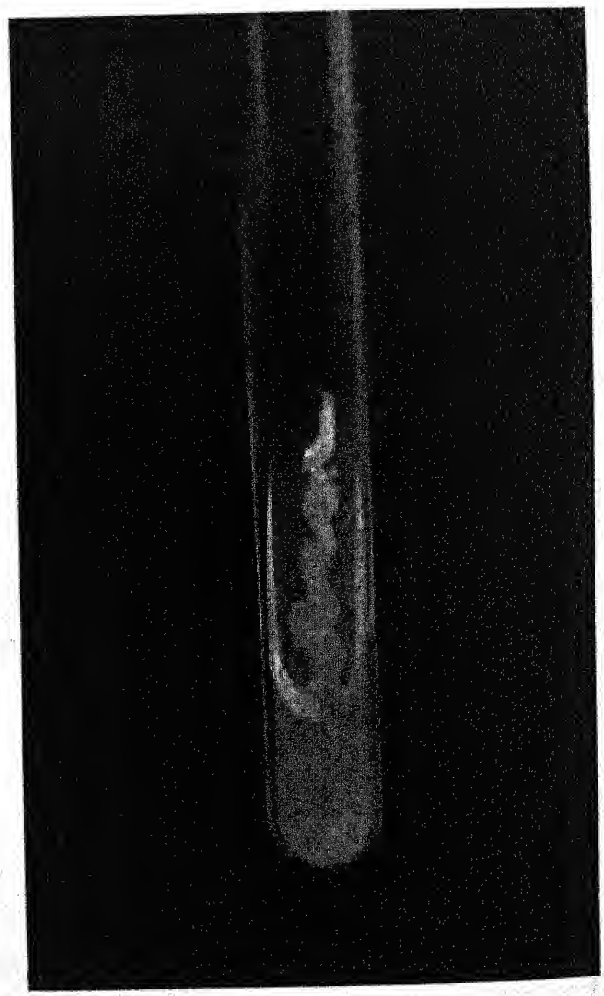


FIG. 3. *C. equi.* 48 hours' growth on agar slant. Growth had begun to flow and gather at the bottom.

faeces. Histologically the pleura of the affected lungs is seen to be infiltrated with epitheloid cells. The interlobular septa are considerably thickened due to fibrinous infiltration and newly formed thin-walled blood vessels. There is evidence of acute bronchitis and peri-bronchitis, the bronchioles containing numerous neutrophiles and cast off epithelial cells held together in a fibrino-mucin: extensive parenchymatous destruction is evident between the lobules, the alveoli are collapsed and their walls are infiltrated with neutrophiles and large mononuclears, the former in various stages of degeneration. The invading organisms are found almost everywhere lying in clumps, enclosed within peculiar giant-cell-like formations. It would appear that when these formations become very numerous in any part, then the lung tissue there undergoes necrosis and the softening of the necrosed tissue gives rise to the pus-like material in the large abscesses to be seen by the naked eye. In advanced stages where the entire lobule presents to the naked eye the appearance of a suppurative lesion, the histological picture presented is one of a mass of necrosed cells with large clumps of the organisms lying about.

Bacteriology

Corynebacterium equi, the causal organism, is recoverable in pure culture and sometimes mixed with other contaminants, particularly streptococci, from the lung abscesses.

The organism is a short cocco-bacillus with the axis straight or slightly curved. Many are found to be almost coccoid in tissue smears and in smears from solid media. They measure from 1·2 to 1·5 μ in length and 1 to 1·2 μ in breadth. In liquid cultures they occur in comparatively longer rods. These may measure up to 3 to 5 μ in length. Sides are usually parallel or slightly bulging, ends rounded. They occur in clumps in tissue smears and singly or in pairs in smears from broth culture. Some show a central or subterminal fusiform enlargement. They are non-motile and non-sporulating. No capsules were demonstrable by the Hiss's copper sulphate method. It is gram-positive but non-acid fast. Stained by Leishman's stain it takes the basic stain and is beaded. The beads may be bipolar and in long rods from broth cultures, they may number as many as 4 or 5 distributed through the length of the rod (Plate IX, Fig. 3). The organism does not take the Neisser's stain well and no metachromatic granules are demonstrable.

On solid media the colonies are circular, about 0·6 to 1·2 mm. in 24 hours but grow considerably bigger by the 24th or 36th hour, measuring as much as 6 mm. They are raised amorphous and have a smooth shining surface. They have an entire margin, are butyrous in consistency and are readily emulsified into a homogeneous emulsion when rubbed down with water. In the first few days they are of a dull-fawn colour but later develop into a brick-red or pink colour (Plate XI). The organism grows abundantly in stroke culture, and is mucoid in appearance, but it does not draw off into a thread when touched by a platinum

loop. Very soon, within 24 to 48 hours, the growth begins to flow down the incline of the agar slope and gathers at the bottom (Plate X, Figs. 2 & 3). In stab cultures growth is confined to the surface.

There is a moderately good growth in broth culture, with a slight uniform turbidity, which is sometimes more marked in the top layer. There is a slight imperfect granular surface ring growth at the junction of fluid and glass and this has a tendency to climb up the wall of the tube on shaking. In long-standing cultures in flasks, islands of granular scum may be seen unevenly distributed over the surface. There is a moderately abundant powdery deposit which takes on a fawn colour with age. The deposit disintegrates almost completely on shaking. No special odour is attached to these cultures. It grows profusely in blood agar, but produces no haemolysis.

Resistance.—The organism is killed by heating a 24 hours broth culture in the water bath at 58° C. for one hour.

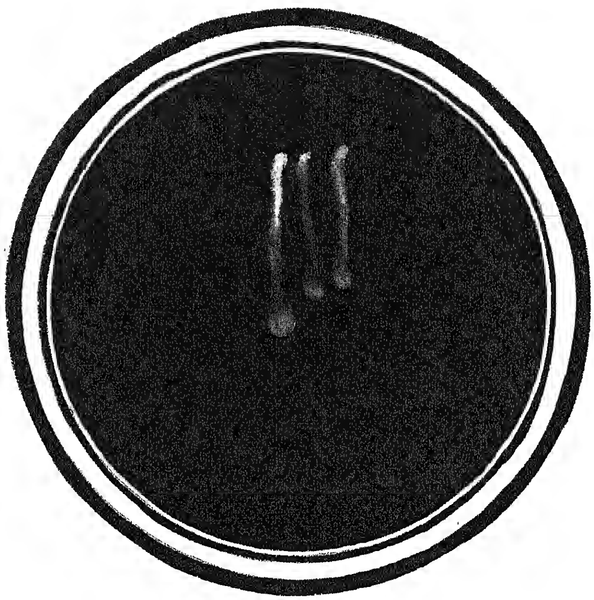
Metabolic properties.—The organism grows best aerobically, and moderately under micro-aerophilic conditions. It does not grow under anaerobic conditions. It forms a brick-red to salmonpink coloration on solid media, particularly when kept exposed to light. Growth on sodium tellurite agar is black in colour. No growth is visible on a MacConkey plate in 24 hours, but in from 2 to 3 days minute streptococcus-like colonies 0·2 to 0·25 mm. in diameter make their appearance. It does not grow in Kesslers' [1926] medium containing 1 in 20 of bile and one in 25,000 of gentian violet, nor does it grow in Salle's [1930] medium containing 1 in 50,000 of crystal violet.

Biochemical reactions.—About 25 carbohydrates consisting of mono-saccharides, di-saccharides, tri-saccharides, poly-saccharides, alcohols, glucosides and the benzolic compound 'Inosite' were tested for fermentation with negative results. Litmus milk is not changed. It is M. R., V. P., indol, ammonia, hydrogen sulphide, and M. B.-reductase negative, and is positive for the catalase test and for nitrate reduction.

Pathogenicity

No toxin was demonstrable in the filtrates. The organism was not pathogenic for Guinea-pigs and rabbits by the intraperitoneal, subcutaneous, intratracheal and intravenous routes. Strangely, two rabbits fed with freshly isolated cultures behaved differently. One of these showed a temperature collapse on the 2nd day after feeding and died that night. The other showed a slight temperature reaction of 103° F. from the 18th day and was destroyed on the 20th day. The lungs of the latter showed a few nodules. *Corynebacterium equi* was isolated from both these rabbits. The results of small animal inoculation are tabulated in Table I.

Several attempts were made to reproduce the disease in ponies and foals. The results are tabulated in Table II.



C. equi : 4 days' growth in agar plate kept in an upright position. Note the pink colouration

TABLE I

Experimental infection of small animals

Animal species	Animal No.	Nature of culture	Dose	Method	Reaction	Agglutination test	Result of experiment	Culture	Lesions
Guinea pigs	290 B	Broth 48 hours	1 c.c.	Intra-peritoneal.	Slight temperature ? 8th to 11th day.	Not tested.	Lived. Discontinued.		
	292			Subcutaneous.	Nil		Lived. Discontinued.	Not examined.	
	222	Agar. Brown 4 opacity.	0·25 c.c.		Nil		Lived. Discontinued.	Sterile.	No lesions.
	223		0·25 c.c.	Intra-tracheal	Nil		Lived. Discontinued.	Sterile.	No lesions.
	169	Broth. 2 days growth	5 c.c.	Drenched	Nil	18th day negative.	Destroyed 28th day.	Blood, Lung } Negative.	
	170	Agar. Brown 4 opacity			Temperature reaction from 18th day.		Destroyed 20th day.	Lung nodules and lung substance pure culture. Blood—Negative.	Nodular lesion without limiting structure. Massed plasma cells in symplasma. Large number of eosinophiles. No organisms detected in gram-stained sections.
Rabbits	171	Broth 2 days growth	5 c.c. daily for 3 days.	Fed.	Nil	Not tested.	Destroyed 19th day.	Blood, Lung } Negative.	
	172				No fever. Temperature collapse on second day.		Died night of 2nd day.	Lung: Pure culture. Blood: Negative	Not examined.
	173	Agar. Brown 4 opacity.			Nil	13th day negative.	Destroyed 29th day.	Lung, Blood } Negative.	
	587		0·5 c.c. to 1 c.c. of Brown 4 opacity.	Intravenously repeated every 6 days.	Rise of temperature to 107 by 2nd day, fed fairly for 8 days.	50 on 22nd day.	Died 29th day after 1st injection and 1 day after last injection.	Heart blood—Pure culture.	Lungs congested. Diarrhoea.
	588		0·5 c.c. of Brown 4 opacity.	Intra-tracheal	Nil.	Not tested.	Destroyed 27th day.	Negative.	Nil.

TABLE II

Animal number	Age	Mode of infection	Body weight		Agglutination			Result	Symptoms and lesions	Culture
			On arrival lbs.	At death or discontinuance	On arrival	10 days after infection	20 days after infection			
Foal 88	Young	Drenched with 1 litre culture.	..	..	Neg.	..	..	Died 14th day.	Pneumonia. Abscesses in the lungs.	Pure culture of *C. equi* from the abscesses.
Foal 93	1 year	Drenched with 1 litre broth culture.	196	..	Neg.	Neg.	Neg.	Lived	Slight temperature from 14th to 19th day only.	Not attempted.
Foal 94	1 year	Drenched with 100 c.c. broth culture.	190	..	Neg.	Neg.	Neg.	Lived	No reaction . . .	Ditto.
Foal 95	1 year	Intravenously 100 c.c. broth culture.	176	..	Neg.	Neg.	Neg. At death negative.	Destroyed 45th day.	Temperature reaction from the 12th day. Swelling of off-hind fetlock from 14th day which extended to the hip accompanied with much pain. The swelling began to decrease by the 33rd day. Suppurative arthritis.	Pus from limb—Pure culture of *C. equi*.
Pony 89	7 years	Drenched with 1 litre culture.	336	..	Neg.	Neg.	Neg.	Lived	No reaction . . .	Not attempted.
Pony 90	5 years	10 c. c. culture intravenously.	294	..	Neg.	Neg.	Neg.	Lived	Slight temperature 10th to 20th day only.	Ditto.
Pony 91	8 years	10 c.c. culture subcutaneously.	368	..	Neg.	Neg.	Neg.	..	..	..
Pony 92	Aged	Control. No inoculation.	360	..	Neg.	Neg.	Neg.	Lived	Nil	Not attempted.
Foal 386	1 month and 3 weeks	Instilled 5 c.c. of a 800 millions per c.c. emulsion of *C. equi* into the nostrils and trotted the foal for ½ an hour.	..	..	Neg.	Neg.	Neg.	Died 18th day.	Temperature of about 101·5 from 5th to the 7th day with watery discharge from nostrils and eyes. Sudden kick in temperature to 106 by the 8th day, which ranged from 102 to 104 until just before death when there was a sudden temperature collapse. Swelling of both hock joints on 11th day which	Heart blood=sterile. Mediastinal gland=Pure culture of *C. equi*. Pus from lungs=*C. equi* and hæmolytic streptococci. Left Hock joint=Sterile. Right hock joint=*Ps. aeuginosa*.

									extended downwards. Symptoms of pneumonia started 9th day, became very aggravated by the 13th day and the animal died on the 18th day. Post-mortem :—abscesses mediastinal gland and lungs. Slightly hæmorrhagic gelatinous semi-coagulated fluid with a few masses of fibrinous adhesions in the right hock joint.	
Pony 342	3 years	Swabbed nostrils with nasal discharges and pus from the lung and mediastinal gland abscesses from foal 386.	..	..	..	..	..	No reaction.	..	..
Foal 382	5 months.	As pony 342 .	..	..	Neg.	Neg.	Neg.	No reaction.	..	..
Foal 387	2 months.	10 c. c. of a 100-million emulsion instilled into nostrils.	82	70	Neg.	Neg.	Neg.	Died 17th day.	Fever temperature 103 on 7th day with cough, increased respiration and pulse. Diurnal variation in temperature from the 10th day. Pneumonic patches noticed in left lung 15th day. Diarrhœa 16th day. Died 17th day.	*During life*: 15th and 16th day blood culture *C. equi.* 16th day—Fæces culture, Pratinum spadeful } Positive for *C. equi.* *After death*: Loopful of heart blood = Negative. Loopful of fæces . = Do. Spleen . = Positive for *C. equi.* Mediastinal gland . = Positive for *C. equi.* Nasal mucosa . = Positive for *C. equi.* Lung abscesses = Positive for *C. equi.*

It will be observed that young foals (*e.g.*, 88, 386, and 287) readily contracted the typical disease with the characteristic lesions after infection both by the oral and nasal routes. It has been relatively difficult or impossible to reproduce the typical disease in older ponies by infection by any route. Nevertheless, it will be observed that two ponies, one after infection by the oral route (pony 93) and the other after infection by the intravenous route (pony 90) developed a slight but undoubted febrile reaction, and that a third pony, after infection by the intravenous route (pony 95) developed a suppurative arthritis in addition to the febrile reaction. *Corynebacterium equi* was re-isolated from the abscesses from the case of suppurative arthritis and from those of typical penumonia. The organism was also recovered from blood and faeces during life and from nasal mucosa, spleen, mediastinal gland and lung abscesses after death from foal 387, the only case in which all these were attempted. It is remarkable, however, that no agglutinins were detectable in the blood serum of any of the infected foals or ponies.

An attempt was made to exalt the virulence of the organism by intra-pulmonary inoculations through rats in series, to see if they could be sufficiently exalted for the production of the typical disease in adult ponies, but no success was obtained by this means.

Prevention

The late Mr. Cooper of this Institute attempted to assess the value of vaccination as a preventive for the disease. Only donkey foals about 6 months old were procurable. A carbolised heated vaccine and a living culture that had been maintained in subculture for a number of years were tried for their immunising properties by subcutaneous inoculations. After an interval of 4½ to 5½ months the vaccinated foals and two control unvaccinated foals were drenched with 1 litre of broth culture sown direct from the pus obtained from the lung of a naturally infected foal. The results are tabulated in Table III.

TABLE III

Donkey Foal No. and age at the time of coming under experiment	Vaccination			Result of test for immunity. On 27th August 1927, each animal was drenched with 1 litre of broth culture sown direct from pus of a natural case
	Preparation and dose	No. and dates of doses	Reaction after vaccination	
43 6½ months.	*Living* diptheroid (*C. equi*) agar emulsion (48 hours growth), 400 mill. per c. c. Subcutaneous 1 c.c.	5 injections monthly from 11th April 1927 to 12th August 1927.	Abscess from each injection.	No reaction.
46 10½ months.			Abscess 1st, 4th and 5th injections.	No reaction.

TABLE III—*contd.*

Donkey Foal No. and age at the time of coming under experiment	Vaccination			Result of test for immunity. On 27th August 1927, each animal was drenched with 1 litre of broth culture sown direct from pus of a natural case
	Preparation and dose	No. and dates of doses	Reaction after vaccination.	
44 6½ months.	Killed. 48 hours agar emulsion of *C. equi* 1600 millions to a c. c. Subcutaneous 5 c. c.	6 injections monthly between 11th March 1927 and 12th August 1927.	First dose was split up into 1 c. c. and 4 c. c. 5 days later. Temperature disturbance. Fed fairly for 3 days. Abscess following 5th injection.	Immediate temperature disturbance and again after one week. 19th day fed fairly, distressed breathing and nasal discharge for 10 days.
45 11 months.			Slight temperature disturbance following 3rd injection.	Immediate temperature disturbance only.
47 1 year 1 month.	Unvaccinated controls.			No reaction except for slight temperature reaction between 55th and 60th days.
50 Not ascertained.				No reaction except for slight temperature reaction between the 22nd and 24th days.

It will be seen that the results are not conclusive, for, at the time of retest even the unvaccinated control foals, along with the vaccinated ones, failed to contract the disease. This is probably due to the fact that the foals were too old. There is, besides, the question whether donkey foals are suitable subjects.

At one stud, which has applied to this Institute for advice, the incidence of this condition, at one time of common occurrence, has been greatly reduced since the introduction of hygienic measures for the control of contagious abortion and the addition of supplements to the diet. Among these measures were included rotation of grazing grounds and paddocks so that they come into use for a year only once in every five years of cultivation, the periodic use of lime, and the doing away with stables, so that foals and mares are in the open day and night throughout the year from date of birth. In addition mares with foals at foot are fed with 20 drops of Tincture iodine every three days.

At the suggestion of Capt. J. F. L. Taylor, R. A. V. C., foals detected at the first rise of temperature are treated in some of the infected establishments with the intravenous injection of 25 c.c. of a 1 per cent solution of mercurochrome, repeated if necessary. In addition the patients are treated with cough electuary and are fed with Tincture iodine in water. The nostrils are douched with a solution of potassium permanganate. Much success is claimed for this line of treatment. A statement showing the results of treatment is attached in Table IV.

TABLE IV

Serial No.	Animal No.	Date of birth	Disease	Date of onset	Treatment Mercurochrome 25 to 35 c. c. of 1 per cent solution intravenously. Tincture iodine—orally Castor oil—orally	Result.
1	Filly 916	19th Septr. 1928	Pneumonia	18th Dec. 1928	Mercurochrome—once	Cured, 31st Dec. 1928.
2	Colt of 917	20th Septr. 1928	Pneumonia	1st Jan. 1929	Mercurochrome—once	Cured, 9th Jan. 1929.
3	Colt of 922	13th Oct. 1928	Pneumonia	1st Jan. 1929	Mercurochrome—once	Cured, 9th Jan. 1929.
4	Colt 931	9th Jan. 1929	Pneumonia	14th Feb. 1929	Mercurochrome—7 injections Tincture iodine—once. Castor oil—once.	Died, 14th March 1929.
5	Filly 926	15th Dec. 1928	Fever	15th March 1929	Mercurochrome—once	Cured, 22nd March 1929.
6	Colt 965	18th March 1929	Pneumonia	26th March 1929	Mercurochrome—once	Cured, 4th April 1929.
7	Filly 932	11th Jan. 1929	Pneumonia	27th March 1929	Mercurochrome—twice	Cured, 3rd April 1929.
8	Colt 945	22nd Feb. 1929	Pneumonia	2nd April 1929	Tincture iodine—thrice Castor oil—twice. Mercurochrome—4 times.	Cured, 17th May 1929.
9	Colt 963	19th March 1929	Fever	18th April 1929	Mercurochrome—thrice	Died, 28th April 1929.
10	Colt 966	20th March 1929	Pneumonia	19th April 1929	Castor oil—once Murcurochrome—twice.	Cured, 4th May 1929.
11	Colt of Welsh Lass.	22nd March 1929	Fever	30th April 1929	Mercurochrome—once	Cured, 8th May 1929.
12	Colt 977	14th April 1929	Pneumonia	12th May 1929	Mercurochrome—twice	Cured, 17th May 1929.
13	Colt 982	28th April 1929	Fever	30th May 1929	Mercurochrome—twice	Cured, 9th June 1929.
14	Colt 968	23rd March 1929	Fever	20th May 1929	Mercurochrome—once	Cured, 23rd May 1929.
15	Filly 933	11th Jan. 1929	Fever	20th May 1920	Mercurochrome—twice Castor oil—once.	Cured, 25th May 1929.
16	Colt of Welsh Lass.	22nd Jan. 1929	Fever	22nd May 1929	Mercurochrome—once	Cured, 25th May 1929.
17	Filly of Silver-cup.	5th April 1929	Fever	22nd May 1929	Mercurochrome—once	Cured, 26th May 1929.
18	Colt 945	22nd Feb. 1929	Fever	22nd May 1929	Mercurochrome—once	Cured, 26th May 1929.
19	Colt 947	23rd Feb. 1929	Fever	21st May 1929	Castor oil—once Mercurochrome—once.	Cured, 26th May 1929.
20	Colt 982	28th April 1929	Pneumonia	30th May 1929	Mercurochrome—twice	Cured, 4th May 1929.

21	Filly of B. M. Silver-cup.	5th April 1929	Pneumonia	30th May 1929	Mercurochrome—twice	Cured, 11th June 1929.
22	Colt 935	21st Jan. 1929	Pneumonia	1st June 1929	Mercurochrome—once Castor oil—once.	Cured, 7th June 1929.
23	985.	3rd May 1929	Pneumonia	7th June 1929	Mercurochrome—once	Cured, 14th June 1929.
24	Filly 951.	26th Feb. 1929	Fever	21st June 1929	Mercurochrome—once	Cured, 28th June 1929.
25	Filly 946	22nd Feb. 1929	Diarrhœa	26th June 1929	Mercurochrome—once	Cured, 19th July 1929.
26	Filly 967	21st March 1929	Fever	21st July 1929	Mercurochrome—twice	Cured, 11th Aug. 1929.
27	Filly 930	5th Jan. 1929	Fever	6th Aug. 1929	Mercurochrome—once	Cured, 9th Aug. 1929.
28	Filly 967	21st March 1929	Pneumonia	17th Aug. 1929	Mercurochrome—twice	Cured, 29th Aug. 1929.
29	Colt 977	14th April 1929	Diarrhœa	19th Aug. 1929	Mercurochrome—once	Cured, 22nd Aug. 1929.
30	Filly 975	11th April 1929	Pneumonia	22nd Aug. 1929	Mercurochrome—once	Cured, 29th Aug. 1929.
31	Colt 974	10th April 1929	Pneumonia	4th Sept. 1929	Mercurochrome—4 times	Cured, 16th Sept. 1929.
32	Filly 979	18th April 1929	Pneumonia	11th Oct. 1929	Castor oil—once Mercurochrome—twice.	Cured, 19th Oct. 1929.
33	Colt 985	3rd May 1929	Catarrh	11th Oct. 1929	Castor oil—once Mercurochrome—twice.	Cured, 19th Oct. 1929.
34	Colt 1003	5th Aug. 1929	Bronchitis	19th Oct. 1929	Mercurochrome—thrice	Cured, 4th Nov. 1929.
35	Colt 1009	14th Aug. 1929	Bronchitis	19th Oct. 1929	Mercurochrome—7 times Tincture iodine—9 times.	Cured, 2nd Dec. 1929.
36	Colt 1007	10th Aug. 1929	Pneumonia	29th Oct. 1929	Mercurochrome—4 times Tincture iodine—9 times.	Destroyed, 28th Nov. 1929.

It may be noted here that out of 37 cases treated, 18 are shown as having developed specific symptoms of pneumonia. Of these 18, fifteen had been cured by adoption of this line of treatment. Of the other 19 animals two were cases of bronchitis, two of diarrhœa and the rest were cases of fever. How many of these cases, if they had been untreated, would have developed into cases of specific pneumonia is unknown. Although, at sight, the adoption of this line of treatment appears to be of benefit, in the absence of adequate controls, it is not possible to assess its true value as a cure for specific pneumonia of foals, particularly as one knows that a certain percentage of affected animals do recover without treatment.

Summary

A particular form of pneumonia is known to occur with some frequency in certain breeding studs in the Punjab, and the etiology of this condition, which was thought to be identical with that described by Magnusson in Sweden, has been under investigation for some time.

The infection is generally confined to foals about one to two months old, and occasionally symptoms of joint-ill may be seen in addition to those of pneumonia. The mortality is high. Post-mortem examination reveals large abscess cavities in the lungs and the mediastinal glands.

Corynebacterium equi, the causal agent, can be recovered in nearly every case in pure culture from the abscesses in the lungs and mediastinal glands, as well as from the faeces, sometimes from the heart-blood and, rarely, from the joint fluid of naturally as well artificially infected cases. The cultural and biochemical characters of this organism are described.

It has been possible to reproduce the typical symptoms of the disease by an intra-nasal douche of a saline suspension of the organism. Age, as in natural incidence, appears to be the chief factor in the artificial reproduction of the disease.

Attempts were made to assess the value of vaccination and of treatment with mercurochrome and iodine. The results were inconclusive for want of animals of suitable age in the former case, and for want of controls in the latter.

Acknowledgments

The late Mr. Cooper took much interest in the investigation of the etiology of this condition and part of the material included in this paper is from some valuable records left by him. I am indebted to Mr. J. R. Haddow for guidance and helpful suggestions and to Mr. V. R. Gopalakrishnan, Veterinary Inspector for assistance in the execution of the work. I have also to thank Capt. Taylor for his permission to record the results of his method of treatment and to Colonel Cole for the supply of pathological materials and certain particulars.

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THE SULPHUR CONTENT OF SOME INDIAN GRASSES

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On account of its possible bearing on wool production the question of the sulphur content of pasture grasses has received a considerable amount of attention, Aitken [1930], Evans [1931], Askew and Bishop [1933], Pollard and Chibnall [1934]. The present short survey originated through an observation made in the course of other work to the effect that grasses of the tribe Chlorideae appear to contain an unusually large amount of sulphate. We have carried out a series of analyses to test this point. Two types of material were collected and examined, *viz.*, natural herbage and cultivated pure grass species. Natural herbage was collected from four distinct types of land. In each place numerous small patches, 1 to 3 sq. ft. in area, were selected and from each area all the specimens of the predominant species were separated and prepared for analysis. Thus it has been possible to compare the nitrogen and sulphur content of various grasses, without the interference of differences in soil and climate, as they were growing side by side in the natural state. Some patches, especially those on richer and more moist soils, yielded five or six species while others, on arid land, yielded only two species. Altogether twenty such patches were sampled botanically and the different species from each patch were analysed separately for total sulphate and total sulphur by our own method [Warth and Krishnan 1935], as well as for total nitrogen. In all about 150 samples were analysed.

Samples of pure species were obtained from plots grown by the Animal Nutrition Section at the Bangalore Dairy. The sampling was done by completely harvesting a small area.

Experimental results

1. *Patches of natural herbage.*—The full figures obtained are too numerous to be quoted here and will be used only for estimating average values. The five following sets of typical data, (actual) given in Table I, in detail, illustrate the nature of the evidence obtained from patches of natural herbage.

TABLE I

Nitrogen and sulphur content of various grasses growing side by side in the natural state

(Expressed as per cent on dry basis)

Area	Grass patch	Name of grass	Total sulphur	Sulphate sulphur	Organic sulphur	Total Nitrogen
North Dairy Plots.	No. 1	*Panicum Crusgalli*	0·203	0·076	0·127	2·080
		Panicum javanicum	0·198	0·060	0·138	2·226
		Digitaria sanguinalis	0·166	0·043	0·123	1·790
		Andropogon annulatus	0·168	0·077	0·091	1·356
		Panicum maximum	0·120	0·032	0·088	1·483
		Eragrostis sp.	0·218	0·095	0·123	1·641
		*Chloris barbata**	0·420	0·289	0·131	1·921
North Dairy Plots.	No. 8	*Andropogon contortus*	0·130	0·069	0·061	0·823
		Andropogon pertusus	0·115	0·045	0·070	1·117
		*Chloris barbata**	0·400	0·290	0·110	1·419
		Digitaria ciliata	0·127	0·052	0·085	1·189
		*Eleusine Indica**	0·286	0·185	0·101	1·274
		Eragrostis sp.	0·155	0·047	0·108	1·156
		Panicum maximum	0·107	0·038	0·069	0·956
South Dairy Plots.	No. 2	*Cynodon dactylon**	0·503	0·345	0·158	2·424
		*Cynodon dactylon**	0·527	0·393	0·134	2·281
		Panicum javanicum	0·205	0·077	0·128	2·252
South Dairy Plots.	No. 5	*Panicum maximum*	0·168	0·068	0·100	1·522
		*Chloris barbata**	0·331	0·218	0·113	1·779
		Panicum javanicum	0·184	0·069	0·115	1·751
Military Grass Farm.	No. 1	*Andropogon pertusus*	0·137	0·055	0·082	1·180
		Andropogon contortus	0·116	0·044	0·072	0·908
		*Chloris barbata**	0·281	0·180	0·101	1·078
		Cymbopogon sp.	0·135	0·058	0·067	1·222
		Eragrostis	0·171	0·052	0·119	1·148
		Panicum javanicum	..	0·061	..	..

Species of Tribe Chlorideae marked *.

The figures show that amongst grasses growing side by side in the natural state, species of the tribe Chlorideae contain much more sulphate than is found in other species. The same fact is borne out equally clearly from all the other patches as well.

2. *The average nitrogen and sulphur content of different species.*—The accompanying table (Table II) is a summary of all our data obtained both from natural herbage and from cultivated plots.

TABLE II

Average sulphur content of grasses. Per 100 parts dry plant

Name of grasses	No. of samples	Total Sulphur	Sulphate Sulphur	Organic Sulphur	Total Nitrogen	Nitrogen : Organic Sulphur
						Ratio
Panicum crusgalli	2	0·188	0·059	0·129	2·160	17
Panicum javanicum	7	0·202	0·068	0·134	2·167	16
Panicum maximum	8	0·123	0·038	0·085	1·353	16
Pennesetum cenchroides	6	0·196	0·077	0·119	1·962	16
Andropogon contortus	6	0·123	0·059	0·064	0·870	14
Andropogon annulatus	4	0·163	0·083	0·080	1·157	14
Andropogon pertusus	1	0·115	0·045	0·070	1·117	..
Sorghum vulgare	1	0·137	0·030	0·107	1·986	..
Holcus sp.	1	0·245	0·031	0·215	1·502	..
Cymbopogon	3	0·106	0·034	0·071	1·064	15
Aristida sp.	2	0·120	0·037	0·083	1·164	14
Eragrostis sp.	6	0·187	0·067	0·120	1·396	12
Rhodes grass	6	0·234	0·129	0·105	1·774	17
Eleusine coracana	8	0·405	0·281	0·124	2·353	19
Eleusine Indica	1	0·286	0·185	0·101	1·274	..
Chloris barbata	8	0·477	0·359	0·118	1·704	14
Cynodon dactylon	5	0·562	0·423	0·139	2·228	16

The last 5 grasses belong to the tribe Chlorideae.

From the results given in Table II, it will be observed that the sulphate content of the cultivated grasses agrees with the results obtained in the case of natural grasses.

The data do not reveal any other specific differences in sulphate content. The low value for *Panicum maximum* appears to be characteristic of this species. The average value recorded here for this grass agrees with the figures obtained from numerous determinations made by us in connection with other work.

The organic sulphur is present, presumably, mainly as protein sulphur, since it fluctuates with the nitrogen content, as the above figures show.

In view of the high sulphate values it is of considerable interest to enquire whether the protein of the Chlorideae is unusually rich in sulphur. The values for the nitrogen : organic sulphur ratio indicate that there is no sign of greater richness of sulphur in the proteins of the Chlorideae. As the nitrogen values refer to the total nitrogen and the organic sulphur includes non-protein sulphur, the above ratio is not an exact measure of protein sulphur, but as far as the figures go, they suggest that the proteins of the Chlorideae do not contain significantly higher amount of sulphur than those of other species. On the other hand it would seem that the proteins of *Eragrostis* may be somewhat richer in sulphur content.

3. *Distribution of sulphate between leaf and stalk.*—The data in Table III show that the percentage of sulphate is higher in the stalk than in the leaf in all the plants examined except lucerne.

TABLE III

Sulphate content of stalks and leaves of grasses. Sulphate expressed as grms. sulphur per 100 parts dry grass

Species	Stage of maturity	Stalks	Leaves
Elusine coracana	flowering	0·362	0·147
Do.	full flower	0·390	0·203
Do.	dead ripe	0·518	0·199
Rhodes grass	full bloom	0·221	0·133
Andropogon contortus	Do..	0·054	0·023
Pennesetum cenchroides	Do.	0·051	0·031
Panicum maximum	Do.	0·044	0·025
Sorghum vulgare	Young	0·026	0·009
Cynodon dactylon	Full bloom	0·326	0·275
Lucerne	Do.	0·099	0·199

From the figures in Table III, it appears to be a general rule with grasses that the stalk contains more sulphate than the leaf. Lucerne apparently behaves differently, and we have another set of figures for the same plant, determined on a different occasion, which also corroborates the above results. The additional figures are given below :—

Sulphate as grams sulphur per 100 grms. dry plant

	Plot 1		Plot 2	
	Leaf	Stalk	Leaf	Stalk
Morning	0·237	0·153	0·219	0·136
Evening	0·191	0·124	0·200	0·127

It may be mentioned that this distribution can only be determined if the leaf and stalk are separated at the time of harvesting. If the plant is allowed to dry in air before leaf and stalk are separated, a large amount of the sulphate of the stalk is drawn into the leaf. The following experiment illustrates this point clearly.

A sample of Guinea grass was cut, one part of which was air-dried before separating leaf from the stalk, and another part was separated and then air-dried.

The distribution of sulphate between leaf and stalk as the result of the above treatment is given below :—

Sulphate as grams sulphur per 100 grms. dry plant

	Leaf	Stalk
Partial drying before separating	0·029	0·037
Separating and then partial drying	0·019	0·044

Summary

Amongst grasses growing side by side in the natural state, it was found that species of the tribe Chlorideae contain more sulphate than is present in other species. There was no corresponding excess of sulphur in the proteins of these species.

The stalks of grasses contain more sulphate than the leaf. In lucerne, the reverse is true.

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IS THERE A RELATIONSHIP BETWEEN THE VIRUSES OF RINDERPEST AND DOYLE'S DISEASE ?

BY

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It was suggested by Kylasam Aiyer[1931] that a relationship exists between the Madras Fowl Pest virus (Doyle's disease virus) and the rinderpest virus. If this were substantiated it would lead to far-reaching developments. It was, therefore, decided to verify the correctness of this supposition. Mr. Ware, the Director of this Institute, laid down the outline of the work to be undertaken in this connection and the experiment was completed during 1931. As no further communication has since appeared on the subject either in support or against the above statement, it is now considered desirable to record the results obtained at this Institute.

The two most reliable methods of establishing identity of viruses are by cross-immunity tests and serum-virus neutralisation tests. It was decided to attempt only cross-immunity tests for this preliminary investigation.

The plan of the experiment was as follows :—

(1) To accommodate two healthy susceptible hill bulls in experimental *chuppers* and inject them each with recently tested virulent Doyle's disease virus. At the same time two susceptible fowls would be injected with the same virus to prove its potency.

(2) To accommodate three healthy fowls—birds which have been remaining in a healthy condition at Muktesar for at least some weeks—in an isolated shed and to inject them with 0·5 c. c. of a good hill bull strain of rinderpest virus. At the same time two bulls would be injected with 5 c. c. of the same virus to prove its potency.

(3) If a reaction occurred after injection of the viruses, then sub-inoculations would be carried out as follows :—

(*a*) If the cattle reacted, blood would be drawn from them and injected into two healthy hill bulls and two healthy fowls.

(*b*) If the fowls reacted mouth washings and blood would be taken and injected into two fowls and two hill bulls for each material.

(4) If no reaction occurred, or any animals survived the inoculation for 10 days, then they would be tested for immunity giving all hill bulls rinderpest virus and all fowls Doyle's disease virus.

THE EXPERIMENT

1.—To test if Doyle's disease virus will give rise to rinderpest in bulls—

Three Indian strains of Doyle's disease virus were available. They had been stored in the refrigerator in the form of organ pulp since 11th May 1931, 11th May 1931, and 20th May 1931, respectively. The strains dated 11th May 1931 had been inoculated into fowls on 23rd May 1931 and found to be virulent. These three viruses were mixed and used for inoculation in connection with this experiment on 26th May 1931. Two susceptible hill bulls, Hill Bull 63 and Hill Bull 111, were each injected subcutaneously with 15 c. c. of a thick emulsion. At the same time two fowls, Fowl 20 and Fowl 21 were injected subcutaneously with 5 c. c. of the same emulsion, to serve as controls for the viability of the virus.

The two fowls used for control died of Doyle's disease on the 29th and 30th respectively of the same month. The virus was, therefore, potent.

Of the bulls inoculated with the Doyle's disease virus, Hill Bull 63 showed no reaction nor did it acquire any immunity against rinderpest as judged by a subsequent re-test 10 days later. But the other bull, Hill Bull 111, showed a sharp rise in temperature on the 4th day after the inoculation, and sub-inoculations were carried out from it. Fresh whole blood was used. Two bulls, Hill Bull 80 and Hill Bull 272 were each injected subcutaneously with 5 c. c. and two fowls, Fowl 25 and Fowl 26, were each injected with 2 c. c. by the same route.

The sub-inoculated bulls developed no reaction and were also shown to be subsequently susceptible to rinderpest when re-tested 11 days later. This points to the conclusion that the blood drawn from Hill Bull 111 contained no virus that will give rise to rinderpest in bulls. Further evidence pointing to the same conclusion was obtained when Hill Bull 111 itself was submitted to a re-test with rinderpest virus on the 11th day after inoculation with the Doyle's disease virus. It developed all the classical symptoms and lesions of rinderpest and died of it.

Of the fowls sub-inoculated from Hill Bull 111, Fowl 25 died on the 5th day, but as it was decided not to conduct any further sub-inoculations in this preliminary experiment, it cannot be affirmed with certainty as to the actual cause of death. There was a coincident helminthic infection and the probability is that it did not die of Doyle's disease. This is borne out by the fact that its pair, Fowl 26, did not develop any symptoms at all and was found to be subsequently susceptible to infection with Doyle's disease virus when tested 10 days later.

The results of this part of the experiment are presented in tabular form in Table I.

TABLE I

Doyle's disease virus.

	Controls for the viability of the virus.		Bulls used for test.					
1. Numbers of the animals inoculated with Doyle's disease virus.	F 20	F 21.	H.B. 111.					H. B. 63.
2. Reaction following the inoculation with Doyle's disease virus.	Fever, fed fairly, dull. Died of D. disease.	Fever, fed fairly, dull. Died of D. disease.		Transient fever, fed fairly.				No reaction.
3. Material used for sub-inoculation				Jugular blood.				
4. Animals used for sub-inoculation.				H. B. 80	H. B. 272	F. 25	F. 26.	
5. Reaction following the above sub-inoculation				No reaction.	No reaction.	No reaction. but died on the 5th day. Helminthiasis ?	No reaction.	
6. Result of re-test:—Bulls with *rinderpest virus*. Fowls with Doyle's disease virus			Fever, off-feed, vesicles, diarrhoea; Died of rinderpest. Positive.	Fever, fed fairly, dull. Positive.	Fever, off-feed, dull, diarrhoea. Positive.		Fever, temp., collapse, off-feed, dull. Diarrhoea. Died of D. D. Positive.	Fever, off-feed, diarrhoea, vesicles. Positive.

(The re-test inoculations shown against column six were done, as a rule, on the 10th or 11th day after the last inoculations.)

This shows clearly that Doyle's disease virus neither gives rise to rinderpest in bulls nor does it confer any protection to bulls against a subsequent infection with rinderpest. It also shows that Doyle's disease is not inoculable into bulls.

II.—To test if rinderpest virus will give rise to Doyle's disease in fowls—

Defibrinated virulent blood was obtained from a hill bull virus producer at the height of the thermal reaction on the 4th day after inoculation. The donor of the virus, Hill Bull 14, later developed the typical symptoms and lesions of rinderpest, including diarrhoea and vesicles. Three fowls, Fowl 22, Fowl 23 and Fowl 24 were inoculated each with 0·5 c. c. At the same time two hill bulls, Hill Bull 115 and Hill Bull 49 were each injected with 5 c. c. of the same blood to serve as controls for the viability of the virus. These bulls developed the typical thermal reaction and diarrhoea of rinderpest and were found to have been rendered immune on a subsequent retest with rinderpest virus. The virus from Hill Bull 14 has thus been shown to have been viable.

Of the three fowls inoculated above with the rinderpest virus, Fowl 23 and Fowl 24 showed no reaction whatever. It was also found that the rinderpest inoculation had conferred no immunity on them against Doyle's disease, for, when retested 10 days later they succumbed to the inoculation. The third fowl, Fowl 22, however, developed a slight thermal reaction and constitutional disturbances such as dullness and inappetence on the 4th day after the inoculation. Sub-inoculations were carried out from this fowl with the mouth washings and blood using bulls and fowls for both the materials.

The mouth washing was inoculated subcutaneously into Hill Bull 229 and Hill Bull 233 at 5 c. c. each and into fowls, Fowl 27 and Fowl 28, at 3 c. c. each. Hill Bull 229 and Hill Bull 233 did not develop any reaction and were found to be subsequently susceptible to rinderpest when retested with rinderpest virus 10 days later. Fowls, F. 27 and F. 28 became dull, fed fairly and had soft faeces, but regained normal health eventually. They were proved to have developed no immunity against Doyle's disease virus, for when they were tested 10 days later with Doyle's disease virus, they developed the clinical symptoms of Doyle's disease and died. This conclusively shows that the ailments of F. 27 and F. 28 following the inoculation of mouth washings from Fowl 22 was not attributable to Doyle's disease.

The whole blood from Fowl 22 was injected subcutaneoulsy into bulls, Hill Bull 248 and Hill Bull 312, at 5 c. c. each and into fowls F. 29 and F. 30 at 3 c. c. each. None of the bulls showed any reaction following this inoculation and among the fowls only F. 29 had slight constitutional derangement. These animals were retested for immunity 10 days later. The bulls were found to be susceptible to rinderpest. The fowls were found to be susceptible to Doyle's disease and they died of the test inoculations.

These results are presented in tabular form in Table II.

Table II

Rinderpest virus from H. B. 14.

	Controls for the viability of the virus.		Fowls used for test									
1. Number of the animals inoculated with the rinderpest virus.	H.B.115	H. B. 49	F. 22.								F. 23	F. 24
2. Reaction following the inoculation with the rinderpest virus.	Fever, fed fairly, dull, diarrhoea.	Fever, fed fairly, dull, diarrhoea.	Fever, fed fairly, dull, destroyed.								No reaction	No reaction
3. Material used for sub-inoculation.			Mouth washing.				Blood.					
4. Animals sub-inoculated.			H. B. 229	H. B. 233	F. 27	F. 28.	H. B. 312	H. B. 248	F. 29	F. 30		
5. Reaction following the above sub-inoculation.			No reaction	No reaction	Fed fairly, soft faeces, recovered.	Fed fairly, soft faeces, recovered.	No reaction	No reaction	Fed fairly, soft faeces, recovered.	No reaction		
6. Reaction following re-test:—Bulls with rinderpest virus and fowls with Doyle's disease virus.	No reaction Negative	No reaction Negative.	Fever, fed fairly, dull, vesicles, soft faeces. Positive	Fever, fed fairly, dull, vesicles, diarrhoea. Positive.	Thermal collapse, off feed, diarrhoea. Died of D. D. Positive.	Fever, thermal collapse, off feed, diarrhoea. Died of D. D. Positive.	Fever, off feed, dull, diarrhoea. Died of rinder-pest. Positive.	Fever, off feed, dull, vesicles, diarrhoea. Positive.	Thermal collapse, off feed, diarrhoea. Died of D. D. Positive.	Fever, off feed, dull. Died of D. D. Positive.	Fever, off fed, dull. Died of D. D. Positive.	Fever. Died the day after the retest inocula-tion of co-incident wormy infes-tation. Positive.

The retest inoculation shown against column six were done, as a rule, on the 10th or 11th day after the test inoculation).

These results conclusively show that rinderpest-virus neither gives rise to Doyle's disease in fowls nor does it confer any protection on fowls against a subsequent infection with Doyle's disease virus.

Summary

It has been shown that rinderpest virus does not give rise to Doyle's disease in fowls and *vice versa* and that no cross immunity occurs between the two viruses.

REFERENCE

Kylasam Aiyer, K. (1931). *Ind. Vet. Jour.* 7, 340.

ABSTRACTS

The diagnosis of mastitis. STARR, L. E., PRESCOTT, T. H., and HUFFMAN, J. (1936). *J. Amer. Vet. Med. Assn.* N. S. **41**, 468.

Based on experience of the systematic examination of a dairy herd, the authors draw conclusions on the comparative value of various recognised tests for the diagnosis of bovine mastitis. The following are the tests considered :—

(1) Physical examination : Each quarter of the mammary gland is examined by manual manipulation. The consistency, size, weight, symmetry and the presence of induration or of fibrosis are noted.

(2) Moak's strip cup test : The cup consists of a metal container over which a 100-120 wire screen (or black cloth) has been incorporated within a concave cover. Clots or flakes in the milk are caught in the mesh of the wire or cloth, where they can be readily noted.

(3) Jensen's brom cresol-purple test : Filter paper impregnated with 0·1 per cent of the dye, dried at low temperature and cut into pieces of 1½ in. by ½ in. are used. Two or three drops of milk from the end of the teat is placed on the paper and the results read immediately. A green-tan colour indicates a normal quarter and a deep purple a diseased quarter.

(4) Hayden's Chlorine test : Five c. c. of silver nitrate solution (1·3415 grm. to 1 litre of distilled water) is measured into a test-tube. Two drops of a 10 per cent potassium chromate solution is added, which produces a brick-red colour. The appearance of a yellow colour on addition of 1 c. c. of milk indicates a positive reaction.

(5) The catalase test of Rossel and Miller : Ordinary glass slides are painted on one side with black enamel paint. A large drop of the milk sample is placed on the mirror surface and one drop of 10 per cent watery hydrogen peroxide is added. The appearance of bubbles in the drop indicates infection.

(6) Hydrogen-ion concentration test : 0·5 c. c. bromthymolblue is used as an indicator in 9·5 c. c. of neutral distilled water to which 0·5 c. c. of the milk sample is added. The *p*H is read off by use of a Hellege comparator. A *p*H above 6·8 indicates abnormality.

(7) Hadley's Rennet test : A solution of rennet is prepared by mixing one part of fresh cheese-maker's rennet to 50 parts of distilled water. One-tenth c. c. of the solution is added to 10 c. c. of the freshly drawn sample. The mixture is thoroughly shaken and allowed to stand at room temperature for one hour. Milk which does not coagulate within the first hour is considered to be abnormal.

(8) Bacteriological examination: Plate counts and examinations for significant micro-organisms are made.

Correlating the results, the authors draw the conclusion that :—

(*a*) physical examination in conjunction with the bromcresol-purple test compares very favourably with other methods of diagnosis.

(*b*) the strip cup and rennet tests are simple and reliable for use by the dairyman.

(*c*) the Chlorine and Catalase tests are very sensitive so that they can be used only as laboratory procedures.

(*d*) the *p*H of the milk is not reliable as a diagnostic agent for mastitis.

(*e*) bacteriological examination is too complicated and expensive for routine control of mastitis. (V. R. R.).

The role of nutrition in prevention of disease in farm animals. FRASER, A. H. H. (1935). *Nutr. Abs. & Rev.*, **5**, 2, 295.

Malnutrition may not only produce deficiency disease directly ascribable to a nutritional cause but may render an animal more susceptible and less resistant to the attacks of bacteria or protozoa or parasitic worms producing endemic or epidemic disease. Nutritional science provides an additional means of disease prevention which in the near future may play an important part in maintaining health, increasing production and decreasing cost in live-stock farming. Proper nutrition for the dairy heifer calf is not only essential in order to get healthy cows yielding wholesome milk in large quantities, but is also economical because otherwise the majority of cows will be sold from the byres on account of sterility, mastitis or tuberculosis before they have reached the age of maximum milk production.

Phosphorus deficiency of pasture is the limiting factor in animal production over large areas of the world, and where cereals and oil cakes are extensively used in more intensive system of farming, calcium deficiency is liable to occur. It is probable that a great deal of the disease attributed by practical men to forced production and high feeding is in reality due to an unrecognised latent deficiency in diet. The importance of iodine and iron in goitre and pining districts respectively is proved, although the deficiency of these minerals is not likely to be the sole cause of these conditions. An uncomplicated deficiency of calcium in pasture has not been reported but it is usually always associated with that of phosphorus and probably other elements.

It seems that wild herbivora and native breeds of cattle are not so liable to mineral deficiency as the imported breeds of high fertility, rapid growth and heavy-milking type, because such functions require more phosphorus than the natural pastures of a large area of the world can afford and therefore the improved breeds may die, lose type or revert to scrub under less favourable conditions. A cow yielding 1,500 gallons in a lactating period will excrete a stone of lime in its milk alone ; a 4 lb. hen laying 250 eggs a year will excrete over 3 lb. of lime in the egg shell alone. Thus the modern high-

producing farm animal has unnatural nutritive requirements and the ordinary farm ration is too often a mere compromise between imperfect nutritional knowledge and economic expediency.

Progressive depletion of minerals in pasture lands exerts quite an apparent influence on the general well-being of the stock resulting in conditions like osteophagia, delayed maturity &c. Speaking generally, the balance between the potential productivity of a breed and the natural fertility of soils is a main factor influencing the distribution of different breeds of domesticated herbivora.

The mineral deficiency can be combated by supplying the deficient mineral element in adequate amount either by direct feeding or indirectly through crops by manuring the soil.

Vitamins, unlike minerals, may be synthesised by the animal body from precursors in the food, and its requirements may and do vary between closely related species. Vitamin deficiencies, as a cause of disease, are of comparatively minor importance in all farm stock, unlike laboratory rodents and human beings and can be ignored in the case of grazing herbivora, but the nearer the conditions under which farm animals are kept to those of the laboratory the more evident the need for vitamins becomes.

If the animal is to leave no progeny, then production in dairy cows, bacon pigs and laying hens can be safely forced to its ultimate limit by the aid of concentrated food, but where an animal is to be used for breeding then it should be fed as nearly as possible on its natural food, and it is highly probable that there may be undiscovered food factors in natural foods such as grass and milk which are of importance in live-stock feeding. Based on the results of a number of natural immunity tests on grazing sheep, Anderson and others suggest the presence of a property in growing grass, which has an influence on the defensive mechanism of the animal's tissues. (R. L. K.)

Allantoin, a constituent of maggot excretions, stimulates healing of chronic discharging wounds. ROBINSON, W. (1935). *J. Parasit.*, **21**, 354-358.

A noticeable feature in the maggot therapy of infected wounds is the rapidity of the healing process, and this seemed to the author to be suggestive of the fact that maggots do not merely feed upon necrotic tissue and destroy bacteria but that they also excrete into the wound some substance which is endowed with special curative properties. Investigation showed that this substance was allantoin, which not only occurs as a constituent of maggot excretions, but is a component of urinary secretions of lower animals and is also distributed among plants, where it is found chiefly in the embryos of seeds.

For extracting allantoin, the author placed several thousand nearly full-grown maggots in glass funnels stoppered with cotton. The maggots were occasionally sprayed lightly with water to facilitate excretion and drainage and the excretions were collected in beakers. From this liquid, allantoin was separated as a crystalline substance and its identity was confirmed by comparing its melting point and crystallographic properties with those of an authentic sample.

In his actual therapeutic experiments, however, the author used a 0·5 per cent aqueous solution of commercial allantoin, this being applied daily to the wounds in the form of soaked gauze dressings. The efficacy of this remedy was particularly tested upon chronic non-healing wounds with unhealthy inactive tissue, and in all such cases, small areas of pinkish granulation tissue could be seen growing in the wounds after the first few applications. The treatment was painless and inexpensive.

The author, however, does not claim that allantoin treatment can be entirely substituted for maggot therapy, in view of the fact that maggots play a definite role by removing dead tissue and pus-forming bacteria. [S. K. S.]

NOTE

FOURTH INTERNATIONAL GRASSLAND CONGRESS

THE Fourth International Grassland Congress is to be held in Great Britain in July 1937, under the Presidency of Professor R. G. Stapledon, C.B.E., M.A., Director of the Welsh Plant Breeding Station and the Imperial Bureau for Herbage Plants, Aberystwyth, Wales. The previous Congresses in this series have been held in Europe and membership was more or less confined to European members, but delegates will come to this Fourth Congress from Great Britain, the British Dominions and Colonies, U. S. A. and numerous other countries members of the International Grassland Congress Association (Central Office in Leipzig, Germany).

The paper-reading sessions will be held in Aberystwyth from July 13th to 19th, but intending participants will be able to join in a tour of centres of grassland interest and selected farms both before and after these sessions. The tour will be made partly by motor coach and partly by rail.

Delegates can choose one of the following options :—

	July
(1) Attending paper-reading sessions and local Aberystwyth tours only	13—19
(2) Assembling at Oxford, proceeding *via.* selected centres of grassland interest to Aberystwyth and participating in sessions and local tours	8—19
(3) Attending paper-reading sessions and local tours, and proceeding from Aberystwyth to Newcastle, and Edinburgh, where disperse.	13—23
(4) Assembling at Oxford, proceeding to Aberystwyth, attending paper-reading sessions and local tours, and proceeding thence to Edinburgh, *i.e.*, the whole tour, of which options, 1, 2 and 3 are only parts	8—23

The tours have been so arranged that participants will have an opportunity to see something of British grassland farming, including live-stock management, over as wide a range as possible. The limits of what can be done have been set by the amount of time available, and the obvious necessity for curtailing charges to delegates.

Special addresses will be given on certain evenings during the course of the tour, when matters of general interest emanating from the tours will be dealt with. These addresses will be given at Oxford, Cirencester, Aberystwyth and Newcastle.

Approximate quotations are now given for the cost of the tours. Participants selecting option (1) will be expected to pay the Congress Fee of two pounds (sterling) but to find their own accommodation in Aberystwyth. A list of approved hotels,

hostels, etc., in Aberystwyth and the neighbourhood can be obtained from the Joint Secretaries. The charges for the other three options are as follows :—

Option (2).—Transport and accommodation in colleges where possible £11. Hotel accommodation can be provided at £13 per head.

Option (3).—Transport, and accommodation in colleges where possible £11. Hotel accommodation can be provided at £13 per head.

Option (4).—The complete tour. Transport, and accommodation in colleges where possible £18. Hotel accommodation can be provided at £21 per head.

Alternative rates can be quoted for those intending to provide for their own transport on tour.

As already stated the Congress Fee for the Fourth Congress is two pounds sterling which will entitle members to attend all sessions and to receive the printed transactions, including all abstracts in advance of the Congress meetings, and any other incidental matter relating to the Congress. The Congress fee for wives accompanying members will be one pound sterling and will admit to full membership but will not entitle such members to receive a copy of the transactions.

The paper-reading sessions to be held in Aberystwyth will be divided into three plenary and two sectionalized sessions. The sectionalized sessions will deal with the following aspects of the grassland problem.

(1) Ecology (including surveys), pasture and range management (including erosion control).

(2) Seeds mixtures (including lucerne for grazing); legumes for use in poor pastures.

(3) Plant breeding, genetics, and seed production.

(4) Manures and fertilizers.

(5) Nutritive value of pastures; fodder conservation.

(6) Grassland economics.

All particulars regarding the acceptance of papers and dates for receipt of abstracts and paper manuscripts may be had from the Joint Secretaries, Agricultural Buildings, Aberystwyth, Great Britain, to whom requests for the Preliminary Programme and application form for membership and all other correspondence regarding the Congress should be addressed.

APPENDIX

INSTRUCTIONS TO AUTHORS OF PUBLICATIONS OF THE IMPERIAL COUNCIL OF AGRICULTURAL RESEARCH*

1. All manuscripts should be clean, clear and carefully revised. Only one side of the paper should be used, and as far as practicable the original type-written copy and not a carbon copy should be sent. Capitals should be sparingly used, and all the necessary punctuation should be done in the MS. and not left for introduction in proofs.

2. The title of a paper should not be lengthy.

3. It is desirable that the MS. should have suitable heads and sub-heads. In numbering the principal divisions of a paper roman numerals should be used. The use of arabic figures and (*a*), (*b*), (*c*), etc., is generally reserved for numbering the sub-divisions coming under each head.

4. Articles submitted for publication either in the *Indian Journal of Agricultural Science* or in the *Indian Journal of Veterinary Science and Animal Husbandary* should be accompanied by abstracts for publication in *Agriculture and Live-stock in India*. Abstracts should be concise, but should be long enough to explain the matter dealt with ; ordinarily no abstract should exceed 200 words.

5. When a word or line is intended to be printed in *italics* it should be underlined with a single line, in SM. CAP. with two lines, in CAPITALS with three lines, and when in **Antique** (heavy type) with a wavy line (~~~~~~~~~~~~~).

6. In descriptive matter, numbers under 100 and all numbers occurring at the beginning of a sentence should be in words.

7. Local names for crops, technical operations, etc., should be defined where they first occur in the text, *e.g.*, *rabi* (spring crop). The use of local weights and measures should be avoided as far as possible. Vernacular names, such as *jowar*, *bajri*, should be in italics without a capital letter, and each such name where it first occurs should be followed by its scientific equivalent in brackets, *e.g.*, *jowar* (*Andropogon Sorghum*). It is usual to write the initial letters of varietal names in capitals, *e.g.*, Striped Mauritius, Dharwar-American cotton and Broach cotton.

*Spare copies of these Instructions can be had on application to the Secretary, Imperial Council of Agricultural Research (Publication Section), New Delhi.

8. Botanical and zoological names are printed in italics and should be underlined in the MS., *e.g.*, *Triticum vulgare* L.; *Diplodia Corchori* Syd.; *Pyrilla aberrans* Kirby. The International rules of Botanical Nomenclature and the International rules of Zoological Nomenclature should be followed. The names of chemical substances should not be written with a capital letter; they are printed in roman type (*e.g.*, calcium carbonate, prussic acid).

9. The following and similar abbreviations may be used freely :—*viz.*, *e.g.*, *i.e.*, mm. (millimetre), cm. (centimetre), grm. (gramme), mg. (milligramme) c. c. (cubic centimetre), sp. gr. (specific gravity), lb. (pound), cwt. (hundredweight), in. (inch), ft. (foot), oz. (ounce), md. (maund), sr. (seer). ch. (chhattak). Other abbreviations should be used sparingly, if at all.

10. References to plates should be given within brackets, without prefixing the word "see" or "cf.", in the MS. itself, and should not be left over for introduction in proofs. For example, "The parasite (Pl. X, fig. 4) was present late in 1906".

11. The word "Table" is preferable to "Statement", and tables should be numbered consecutively in roman figures. Each table should have an explanation as a sub-head. It is more convenient for reference if tables can be printed horizontally; for this purpose they should not exceed in width the printing measure of the page (5″). *Example*—

TABLE IV

Results of water-saving experiments on wheat (Pusa 12) at Gungapur, Haripur and Sargodha, 1916-17

Station	No. of irrigations including the preliminary watering	Yield per acre in maunds and seers		Average yield per acre	
		Grain	Straw	Grain	Straw
		Mds. Srs.	Mds. Srs.	Mds. Srs.	Mds. Srs.
Gungapur	One	12 19½	20 10	9 34	21 17
Haripur	,,	8 31	19 14		
Sargodha	,,	8 12½	25 27½		

12. References to literature, arranged alphabetically according to authors' names, should be placed at the end of the article, the various references to each author being arranged chronologically. Each reference should contain the name of the author (with initials), the year of publication, the abbreviated title of the publication, volume and page. In the text the reference should be indicated by the

author's name followed by the year of publication enclosed in brackets; when the author's name occurs in the text, the year of publication only need be given in brackets. If reference is made to several articles published by one author in single year, these should be numbered in sequence and the number quoted after the year both in the text and in the collected references. This system of referencing is used in the *Biochemical Journal* with slight modification, and will be clear from the following illustration :—

The work of Osborne and Mendel [1919, 1, 2] and Steenbock and Boutwell [1919] had indicated an association of the fat-soluble vitamin with the green parts of plants. This view was examined by Coward and Drummond [1921], who reported that vitamin A was not synthesised by etiolated shoots but that green leaves were active in its formation. Another worker [Wilson 1922], on the other hand, found that etiolated shoots if given in sufficient quantity could supply the fat-soluble vitamin and that this factor was therefore formed in the absence of light.

REFERENCES.

Coward K. H. and Drummond J. C. (1921). *Biochem. J.* **15**, 530
Osborne T. B. and Mendel L. B. (1919, 1). *J. Biol. Chem.* **37**, 187.
——————(1919, 2). *J. Biol. Chem.* **41**, 549.
Steenbock H. and Boutwell R. (1919). *J. Biol. Chem.* **41**, 149.
Wilson J. (1922). *J. Biol. Chem.* **51**, 455.

Abbreviations, as far as possible should follow the system adopted in "A World List of Scientific Periodicals" published by the Oxford University Press.

13. Papers should be complete when submitted for publication. As alterations and additions at the proof stage cause both additional expense and delay, they should be resorted to as little as possible. In making corrections in proofs the recognised symbols which will be found in the "Standard Dictionary" should be used. Second (page) proofs will be submitted to authors who should return them promptly.

ILLUSTRATIONS

14. As the *format* of the journals has been standardized, the size adopted being crown quarto (about $7\frac{1}{8}'' \times 9\frac{5}{8}''$ cut), no text-figure, when printed, should exceed $4\frac{1}{2} \times 5$ inches. Figures for plates should be so planned as to fill a crown quarto plate—the maximum space available for figures being $5\frac{3}{4} \times 8$ inches exclusive of that for letterpress printing.

15. Photos or drawings for illustration should accompany the manuscript and each should bear on the reverse side the name of the paper to which it relates together with the title or legend, figure or plate number, and the size to be reproduced. When giving instructions for reduction linear measurements are understood; thus "half-size" means reduce to half the length and breadth, not half the area. A photograph should not be rolled up, nor pinned, and should always be packed flat. A complete list of plates and figures should always accompany the paper.

16. Line drawings should be made with clear black lines on smooth white paper, preferably Bristol board. Rough paper should be avoided. Care should be taken that all the lines are drawn firmly ; scratchy or grey lines, produced by the ink being thinned down, are not permissible. Drawings should be larger than the required size. All lettering should be neatly and clearly put in, care being taken to make all lettering sufficiently large to stand reduction.

17. For half-tone work copy should be made on glossy silver paper and of the same size or larger than the size required.

18. For three-colour work, copy may be oil painting, water-colour, coloured photograph or coloured transparency, and larger than the size required. In preparing copy, one should use only the primary colours, in any combination, as only inks of primary colours are used in printing. Originals can be enlarged, if necessary, but this should be avoided if possible.

19. For detailed instructions regarding preparation of illustrations, it would be of advantage to refer to Mr. C. M. Hutchinson's article on "Photographic Illustrations" in the *Agricultural Journal of India*, Vol. XI, Pt. 3, July 1916, and Mr. A. W. Slater's paper on "The Preparation and Reproduction of Scientific Illustrations" in the *Proceedings of the Third Entomological Meeting* 1919, which has been reprinted as *Bulletin No. 114 of the Agricultural Research Institute, Pusa.*

ORIGINAL ARTICLES

GENETIC PRINCIPLES AND THE PROBLEM OF CROSS-BREEDING FOR MILK YIELD IN INDIAN CATTLE

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Introduction

Numerous attempts have been made in India to combine the milk yield of Western dairy cattle with the hardiness and suitability to local conditions of Indian breeds. It is not proposed in this note to attempt any form of summary of the results of these efforts, but rather to put forward for the consideration of Indian stock-breeders certain genetic principles which appear to bear on the problem,

The aim

Indian cattle have been subjected to countless generations of rigorous natural selection for ability to withstand singularly hard conditions, and no one would dispute the view that for hardiness, vigour, size, conformation, and suitability for draft purposes, the desired type can best be obtained by selection within the indigenous breeds. The principal character of value under Indian conditions in which Western breeds excel the indigenous cattle is milk yield.

Two practices, besides straight selection, are in vogue at present among breeders who are attempting to improve Indian milking cattle. The first, and much the commoner, is to grade up a herd of indigenous cows by repeated breeding back to Western bulls. This is the predominating policy of the military dairy farms. The second is the one which the experiment reported by Littlewood [1933] was designed to test, namely the inter-breeding of cross-bred cattle in an attempt to establish a new intermediate strain. Littlewood [1933] also reports

on the performance of a few animals obtained by breeding the cross-breds back to the Indian breed. This constitutes a third method. The inter-breeding method appears to have been entirely abandoned in India and we are not aware of any sustained attempt having been made to grade up by breeding back to indigenous cattle, though this is the method advocated by Littlewood [1933] as a result of his experience.

An examination of the three breeding practices enumerated above shows that two of them are designed to incorporate in the intended new Indian milking breed more of the Western character than is wanted. Continued grading to Western bulls will result in a strain carrying only such Indian characteristics as are maintained by selection, either natural or human. Inter-breeding will also result in the incorporation of many unwanted Western characters, unless selection is practised of an intensity which the slow-breeding habits of the cow renders a practical impossibility. There remains the method of breeding back to the indigenous type. This method will result in a progressive approach to the indigenous type in all attributes on which selection for the Western character is not practised. Selection need only be practised for characters influencing milk yield, and the object of the experiment becomes simply that of breeding an almost purely Indian type, with a high milking capacity. This, we believe, is the proper aim for those who wish to improve the milk production of the cattle of the country, and not simply to provide specialised milking animals to be maintained by large dairies under special conditions.

The problem

There is a very prevalent opinion that cross-breeding schemes, to have any hope of success, must be large, and consequently expensive. It was expressed by Littlewood [1933] as follows :—" In an experiment of this kind..............at least 200 F_2 cows are necessary in order that a good selection can be made." And later : " I consider it is possible to evolve a half-bred breed of milch cows for India, but it will be an expensive experiment as large herds must be maintained .... ". This opinion appears to be based on the belief that the larger the numbers available the more rigorous the selection that can be exercised. In the long run, however, the intensity of selection is limited, not by the number of animals available, but by the proportion of the young stock which must be retained in order to maintain the strength of the herd. It may, of course, be argued that in the early segregating generations, selection so rigorous as to result in serious depletion of the herd should be exercised, and the herd built up again by relaxation of the selection when a large proportion of the desirable characters have been fixed. Where large numbers of genes are concerned in the make-up of the characters under selection, such a policy would be open to grave objections on genetic grounds, and in any case it is likely that the herd would be extinguished before the desired homozygosity was obtained. This limitation on the intensity of

selection is admirably summarised by Lush [1935], and he concludes that fifty to sixty per cent of the heifers bred must be retained for replacement alone, in a dairy herd. The actual number of animals available, therefore, does not limit the selection intensity, and there is, in fact, much to be said in favour of a herd of moderate size and limited financial demands. India has suffered severely from the abandonment, on grounds of economy, of promising breeding schemes before they had gone far enough to be of practical utility. For this reason also, improvement by cross-breeding has a considerable advantage over improvement by selection, in that there is an immediate and considerable rise in milk yield, which enables a cross-bred herd to pay for its keep, where an indigenous herd frequently does not.

Two factors, which depend largely on the design of the experiment, influence the efficiency of selection. The first is the number of characters for which selection is exercised. Consider for the moment heifers alone, of which fifty per cent must be retained to maintain the herd. If selection is exercised on five independent characters of equal importance, the intensity of the selection exercised on each character is no greater than if the proportion to be retained were $\sqrt[5]{0{\cdot}5}$, or eighty-seven per cent [Lush, 1935].

To put it in another way, only half the heifers can be discarded, or the herd will be depleted. Obviously it is not possible to discard the worst half on all five characters and it will only be possible actually to discard the worst thirteen per cent—a very lenient culling—of each character under selection. $13\times 5 = 65$, and the difference between this and fifty per cent is accounted for by the fact that of those animals which appear in the worst thirteen per cent on one character, some will also appear in the worst thirteen per cent for other characters also.

In the method of back-crossing here advocated, the number of characters for which selection must be exercised is reduced to a minimum, since all characters desired from the indigenous breed are fixed automatically by the back-crossing process without selection, and the selection intensity for the desired Western characters is, therefore, at a maximum.

The second factor in design to be considered, is that selection among males is much more rigorous than among females, since among bull calves the replacement percentage is probably not more than five to ten. Consequently, any breeding method which includes the use of cross-bred bulls will permit of a correspondingly improved selection intensity. This is, of course, offset by the difficulty of evaluating males for the most important characters, namely milk-yield and butter-fat content.

It is beyond the scope of the present paper to advocate particular foreign breeds, but a word must be said concerning the choice of matings. It is a sound breeding policy to avoid introducing variability unnecessarily with regard to

characters in which the breeder is not particularly interested. For instance, the size and type of animal required can usually be decided beforehand from a consideration of the conditions of the tract for which the scheme is to be carried out, and a suitable indigenous breed selected. The foreign breed with which it is to be crossed should not differ more than necessary from it in those characters. Such matings as Sindhi × Holstein are likely to introduce far more variability in size and form and butter-fat content than, for example, Hariana × Holstein-Freisian or Sindhi × Jersey, and will consequently take longer to breed back to a uniform type in which inter-breeding can be started, and from which bulls can be provided for the improvement of cultivators' cattle.

A SUGGESTED BREEDING SCHEME

F_1

It should be remembered that the F_1 is not a segregating generation, and F_1 animals will only differ in those characters, genes for which were heterozygous in one or both parental stocks. As such differences may be expected to be unimportant relative to the differences between the breeds, no selection need be exercised among F_1 heifers.

Since sex is determined by the distribution of the sex chromosome, the above statement must be modified if any sex-linked genes for milk production are carried by the sire. The F_1 generation will then be of two types, heifers which will have received sex-linked genes for milk production from their sire, and bulls which will not have received them.

Evidence for sex-linkage of some of the genes responsible for milk production has been summarized by Smith and Robison [1933], who conclude that it is very suggestive, but not conclusive. Of the bulls used in the breeding of Western cattle breeds some have been what is known as 'prepotent sires' and the exploitation of these has led to their contributing a disproportionate share, compared with other bulls, to the ancestry of the breed. Since prepotency for milk production results largely from a bull's ability to transmit his dam's excellence to his daughters, the fact that all daughters receive a sex-chromosome from their paternal grand-dam and only half receive any given autosome from her will result in stronger selection for any genes for milk production situated in the sex chromosome, than for those situated in autosomes. It is, therefore, to be expected that a disproportionate share of the genes for milk production carried by breeds heavily selected for that character will be sex-linked.

The importance of this conclusion for a cross-breeding scheme with Indian cattle is that, if the effects of sex-linkage can be safely ignored, F_1 bulls are genetically as valuable as F_1 heifers for the production of the first back-cross (or quarter-

bred) generation, and practically, they are much more valuable, since they can sire many calves, whereas each heifer can produce only say six or eight calves in her life. From the summary by Smith and Robison [1933] we conclude that in the Western breeds examined the proportion of milk-yield from sex-linked genes to that from autosomal genes is not high enough to demonstrate their existence with certainty. If that is so where the genetic variance for milk production is comparatively small (*i.e.*, within breeds) it is likely to be at most a very small proportion of the whole in a cross between two breeds differing widely in milking capacity, so that, even allowing for sex-linkage of milk production genes to the extent suggested by Smith and Robison's data, the loss resulting from the use of F_1 bulls in a breeding project of the type under discussion is likely to be negligible.

We suggest, therefore, that both sexes of the F_1 generation should be bred back to the indigenous breed. Then all the available females in the herd can be made to contribute to the back-cross generation, and the maximum number of animals will be provided for selection. A direct check on the accuracy of the above estimate of the importance of sex-linkage in the inheritance of milk yield will then be possible. Since both grand-dams of all back-cross heifers are of the indigenous breed, a figure $\varkappa$ can be calculated for each animal representing the increment in milk yield over the mean yield of its two grand-dams. If sex-linkage is important, the mean of $\varkappa$ for heifers with F_1 dams will be significantly greater than the mean of $\varkappa$ for heifers with F_1 sires.

First back-cross or quarter-bred

This is the first segregating generation, and selection must, therefore, be exercised. We suggest that all quarter-bred bulls should be eliminated from the herd, since the performance of their dams (in a non-segregating generation) will provide only a negligible amount of information concerning their value. All heifers will be bred back to a good bull of the indigenous breed, and selections made on the basis of milking performance. Two points should be noted: first the importance of the indigenous bull. The fact that high milking capacity is being introduced from the Western breed does not release the breeder from the necessity of using the best available indigenous stock. What little is known concerning interaction between genes in quantitative inheritance suggests that genes causing small increments in yield in a low-yielding strain may cause much greater increments in strains of higher milking capacity [Smith and Robison, 1931]. Secondly, the importance of selecting the quarter-bred heifers on milk production alone. The process of further back-crossing will be relied upon to breed out any disadvantageous characteristics which may be carried by good milking animals.

It is not possible to lay down in advance the intensity of selection to be exercised among heifers in each generation, but it should approach on the average that which will allow maintenance of the strength of the herd, and very little more.

Second back-cross or eighth-bred

The second back-cross animals will resemble the indigenous breed closely in most respects. The heifers should be bred back again to an indigenous bull, and selected as in the quarter-bred generation. The second back-cross bulls may be relied on to sire calves almost always superior to those of Indian breeds bearing no foreign ' blood.' They may, therefore, be used in a village cattle improvement scheme, and if suitable arrangements are made for estimating the value of their progeny, the best of them can be picked out and brought back into the herd.

Later generations

Heifers of the third back-cross generation should also be back-crossed to the indigenous breed, and selected on milking performance. As soon as bulls of the second back-cross generation have been tested (on village cattle), the best of them may be brought in and mated to the selected cows of the third back-cross generation. Thereafter, inter-breeding will proceed among heifers selected in each generation on their own milk yield and bulls selected on the performance of their progeny out of village cows.

It should be remembered that when inter-breeding is commenced, all characters of importance will have to be observed, and those besides milk yield which prove not to have been fixed by the back-crossing process will have to be fixed by selection.

GENERAL CONSIDERATIONS

Six quarter-bred cows whose performance is recorded by Littlewood [1933] averaged 3,995 lbs. of milk per lactation as against an average of 4,068 lbs. by nineteen F_2 cows. The difference is negligible, so that with the advantage in favour of the quarter-bred that the whole of the selection can be concentrated on milk yield, the production average of the parents of a second back-cross should be considerably higher than that of the parents of an F_3.

The available evidence indicates that the heterozygotes of milk-yield factors are on the average nearly intermediate between the homozygotes. (The Mt. Hope Index gives 0·7 [Goodale, 1927]). The inter-breeding of the best of the third back-cross animals should, therefore, result in the production of a proportion of heifers with a higher milking capacity than their parents. The advantages of milk production in the first generation which cross-breeding gives over selection in an indigenous herd is increased in the breeding scheme here discussed by the increment in yield obtainable by inter-breeding at the end of the period of back-crossing.

The fear is sometimes expressed that cross-breeding between widely different types will result in genetic incompatibility, and consequently weakly and unbalanced animals. The risk of such misfortunes is minimised by the back-crossing method, as it ensures the re-building of a balanced indigenous type, with only a minimum of introduced genes. Care must be taken to watch for evidence of linkage (repulsion) between milk-yield genes and genes of the indigenous breed which would otherwise be automatically fixed. If such are detected, the breeder must himself decide whether they are of sufficient importance for him to direct his selection towards the discovery and propagation of cross-over individuals, or whether he can afford to allow some deviation from the indigenous type in characters other than milk yield.

This breeding scheme is aimed primarily at providing superior cattle which can be relied on to improve cultivators' stock. It is, therefore, important to decide at what stage untested bulls can be put to village cows with confidence, not merely that on the average their effect will be beneficial, but that there is no greater risk than with good indigenous stock that any individual will have a deleterious influence. If the best available indigenous stock is used in the breeding scheme, second back-cross bulls, selected for vigour and conformation similar to that of their indigenous sires should be safe for distribution. At that stage it is suggested that bulls should be tested for the milk production of their daughters, and the best of them used for inter-breeding with the best third back-cross heifers. Given the confidence of the cultivators and provided a simple method can be devised of estimating the yield of their cows from periodic test milkings, the testing, and the subsequent return to the herd, of the best bulls should be a practical proposition.

In conclusion, it may be remarked that the breeding method here outlined is but a modification for Indian conditions of the methods of grading up adopted with great success in North and South America, South Afri a, and elsewhere. The exceptionally hard conditions in India make it necessary to grade up to the indigenous instead of to the Western breed, but there is no reason why this modification should affect the chances of success. The cattle-breeders of the great new cattle countries have used the best bulls in the world regardless of cost. If this was worth while when the ground lost by the accidental introduction of an inferior animal could be regained by replacement with a better, it is much more important that the best possible Western bulls should be used to start such a scheme, as is here suggested, where Western blood will be introduced once only.

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A CHECK-LIST OF THE NEMATODE PARASITES OF THE DOMESTICATED ANIMALS IN BURMA

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I. Introduction

Although attempts have been made to record the nematode parasites of the domesticated animals in Burma by Evans and Rennie [1910], Gaiger [1910, 1915] and quite recently by Bhalerao [1935], yet our knowledge of these parasites is far from being complete. The present article is yet another attempt not only to place on record an up-to-date knowledge of these parasites, but also to encourage collection of these parasites by those who have ample opportunities of obtaining material, as a great deal of collecting will have to be undertaken before our knowledge of these parasites can be considered anything like complete.

Unless specified, all the parasites listed in this article were identified by the author not only from the collections of the Veterinary Research Laboratory, Insein, Burma, which were in a good state of preservation, but also from large numbers of fresh parasites collected by him in the course of his post-mortem examinations on many experimental animals. The host animals considered in this article are chiefly mammals, *viz.*, elephant (*Elephas indicus*), cattle (*Bos indicus*), buffalo (*Bos bubalus*), sheep (*Ovis aries*), goat (*Capra hircus*), horse (*Equus caballus*), mule (*Equus asinus* × *Equus caballus*), dog (*Canis familiaris*), cat (*Felis catus* var. *domesticus*), and pig (*Sus cristatus*).

II. Class NEMATODA Rudolphi, 1808, emend. Diesing, 1861.

Family Ascaridae Baird, 1853.

Genus *Ascaris* Linnaeus, 1758.

Ascaris lumbricoides Linnaeus, 1758.

Syn., *Ascaris suum* Goeze, 1782.
Ascaris ovis Rudolphi, 1819.
Ascaris suilla Dujardin, 1845.

Host. Pig.

Situation. Small and large intestines.

First record of this parasite from Burma.

Ascaris vitulorum Goeze, 1782.

Host. Cattle.

Situation. Small intestine.

Recorded by Bhattacharjee, 1930 [Mitchell, 1931].

Genus *Parascaris* Yorke and Maplestone, 1926.

Parascaris equorum (Goeze, 1782).
Syn., *Ascaris equorum* Goeze, 1782.
Ascaris equi Schrank, 1788.
Ascaris megalocephala Cloquet, 1824.
Host. Horse.
Situation. Small intestine.
First record of this parasite from Burma.
Genus *Toxocara* Stiles, 1905.

Toxocara canis (Werner, 1782).
Syn., *Lumbricus canis* Werner, 1782.
Ascaris marginata Rudolphi, 1802.
Belascaris marginata Railliet and Henry, 1911.
Host. Dog.
Situation. Oesophagus, stomach, small intestine.
First record of this parasite from Burma.

Toxocara lonchoptera (Diesing, 1851).
Syn., *Strongylus elephanti* Rudolphi, 1819.
Ascaris lonchoptera Diesing, 1851.
Belascaris lonchoptera Leiper, 1911.
Host. Elephant.
Situation. Bile-ducts.
Recorded by Evans and Rennie [1910]. Evans [1910].
Gaiger [1910], Bhalerao [1935]. None in the laboratory collection.
Family Oxyuriidae Cobbold, 1864.
Genus *Oxyuris* Rudolphi, 1803.

Oxyuris equi (Schrank, 1788).
Syn., *Trichocephalus equi* Schrank, 1788.
Lepturis curvula Schlotthauber, 1860.
Oxyuris curvula Rudolphi, 1803.
Oxyuris mastigodes Nitzsch, 1857.
Host. Horse.
Situation. Caecum, colon.
Also recorded by Gaiger [1910], Bhalerao [1935].
Family Trichuridae Railliet, 1915.
Genus *Trichuris* Roederer, 1761.

Trichuris ovis (Abildgaard, 1795).
Syn., *Trichocephalus ovis* Abildgaard, 1795.
Trichocephalus affinis Rudolphi, 1802.
Trichuris affinis Rudolphi, 1802.
Host. Cattle, sheep, goat.
Situation. Caecum, colon.

Trichuris ovis (Abildgaard, 1795)—*cont'd.*

Recorded by Bhattacharjee, 1930 [Mitchell, 1931].

Family Ancylostomidae (Looss, 1905).

Genus *Ancylostoma* (Dubini, 1843).

Ancylostoma caninum (Ercolani, 1859).

Host. Dog.

Situation. Small and large intestines.

Recorded by Bhattacharjee, 1930 [Mitchell, 1931].

Genus *Bathmostomum* Railliet and Henry, 1909.

Bathmostomum sangeri (Cobbold, 1879).

Syn., *Dochmius sangeri* Cobbold, 1879.

Uncinaria sangeri Cobbold, 1879.

Uncinaria os-papillatum Piana and Stazzi, 1900.

Host. Elephant.

Situation. Caecum, intestine.

Recorded by Gaiger [1910], Bhalerao [1935]. None in the laboratory collection.

Genus *Grammocephalus* Railliet and Henry, 1910.

Grammocephalus varedatus Lane, 1921.

Syn., *Grammocephalus clathratus* (Baird, 1868).

Nematode No. 1. Evans and Rennie, 1910.

Grammocephalus intermedius Neveu-Lemaire, 1924.

Host. Elephant.

Situation. Bile-ducts.

Recorded by Evans and Rennie [1910], Khalil [1922], Bhalerao [1935].

None in the laboratory collection.

Family Metastrongylidae Leiper, 1908.

Genus *Dictyocaulus* Railliet and Henry, 1907.

Dictyocaulus filaria (Rudolphi, 1809).

Syn., *Strongylus filaria* Rudolphi, 1809.

Host. Goat.

Situation. Bronchi.

Recorded by Giles, 1892, [Bhalerao, 1935].

None in the laboratory collection.

Dictyocaulus viviparus (Bloch, 1782).

Syn., *Strongylus micrurus* (Mehlis, 1831).

Host. Cattle.

Situation. Bronchi.

Recorded by Bhattacharjee, 1930 [Mitchell, 1931].

Family Strongylidae Baird, 1853.

Genus *Choniangium* Railliet, Henry and Bauche, 1914.

Choniangium epistomum (Piana and Stazzi, 1900).

Syn., *Sclerostoma epistomum* Piana and Stazzi, 1900.

Asifia vasifa Lane, 1914.

Host. Elephant.

Situation. Large intestine.

Also recorded by Meggitt, 1933 [Smith, 1933].

Genus *Decrusia* Lane, 1914.

Decrusia additicta (Railliet, Henry and Bauche, 1914).

Syn., *Strongylus additictus* Railliet, Henry and Bauche, 1914.

Decrusia decrusi Lane, 1914.

Host. Elephant.

Situation. Large intestine.

Also recorded by Meggitt, 1933 [Smith, 1933].

Genus *Equinurbia* Lane, 1914.

Equinurbia sipunculiformis (Baird, 1859).

Syn., *Sclerostoma sipunculiforme* Baird, 1859.

Cylicostomum sipunculiforme Railliet, Henry and Bauche, 1914.

Nematode No. 4. Evans and Rennie, 1910.

Host. Elephant.

Situation. Intestine, caecum.

Also recorded by Evans and Rennie [1910]. Railliet, Henry and Bauche, [1914]. Meggitt, 1933 [Smith, 1933], Bhalerao [1935].

Genus *Murshidia* Lane, 1914.

Murshidia falcifera (Cobbold, 1822).

Syn., *Strongylus falcifer* Cobbold, 1882.

Nematode No. 3. Evans and Rennie, 1910.

Host. Elephant.

Situation. Caecum.

Also recorded by Evans and Rennie [1910]. Railliet, Henry and Bauche, [1914]. Meggitt, 1933 [Smith, 1933], Bhalerao [1935].

Murshidia indica (Ware, 1924).

Syn., *Pterydopharynx indica* Ware, 1924.

Host. Elephant.

Situation. Caecum.

Also recorded by Meggitt, 1933 [Smith, 1933].

Murshidia murshida Lane, 1914.

Host. Elephant.

Situation. Caecum, large intestine.

Also recorded by Meggitt, 1933 [Smith, 1933].

Genus *Oesophagostomum* Molin, 1861.

Oesophagostomum columbianum (Curtice, 1890).
Host. Cattle, sheep, goat.
Situation. Caecum, large intestine.
Also recorded by Giles, 1892, [Bhalerao 1935]. Bhattacharjee, 1930 [Mitchell, 1931].

Oesophagostomum radiatum (Rudolphi, 1803).
Syn., *Strongylus inflatum* (Schneider, 1866).
Oesophagostomum dilatatum (Railliet, 1896).
Oesophagostomum vesiculosum Ratz, 1898.
Oesophagostomum bovis Schnyder, 1906.
Host. Cattle, buffalo.
Situation. Large intestine.
Recorded by Bhattacharjee, 1930 [Mitchell, 1931].

Oesophagostomum venulosum (Rudolphi, 1809).
Syn., *Strongylus venulosus* Rudolphi, 1809.
Host. Goat.
Situation. Large intestine.
First record of this parasite from Burma.

Genus *Quilonia* Lane, 1914.

Quilonia renniei (Railliet, Henry and Joyeux, 1913).
Syn., *Evansia renniei* Railliet, Henry and Joyeux, 1913.
Nematevansia renniei Ihle, 1919.
Quilonia quilona Lane, 1914.
Host. Elephant.
Situation. Caecum, large intestine.
Also recorded by Evans and Rennie [1910]. Railliet, Henry and Joyeux, [1913]. Meggitt, 1933 [Smith, 1933], Bhalerao [1935].

Quilonia travancra Lane, 1914.
Syn., *Evansia travancra* Railliet, Henry and Bauche, 1915.
Nematevansia travancra Ihle, 1919.
Host. Elephant.
Situation. Caecum, large intestine.
Also recorded by Meggitt, 1933 [Smith, 1933].

Genus *Strongylus* Mueller, 1780.

Strongylus equinus Mueller, 1780.
Syn., *Sclerostomum equinus* Looss, 1900.
Strongylus armatus Rudolphi, 1802.
Sclerostomum quadridentatum Sticker, 1901.
Host. Horse, mule.
Situation. Caecum, colon.
Also recorded by Gaiger [1910, 1911]. Boulenger [1921], Bhalerao [1935].

Genus *Trichonema* Cobbold, 1874.

Trichonema aegyptiacum Railliet, 1923.
Syn., *Strongylus tetracanthum* Mehlis, 1831.
Host. Horse, mule.
Situation. Colon.
Also recorded by Giles, 1892 [Bhalerao, 1935].
Family SYNGAMIDAE Leiper, 1912.
Genus *Syngamus* V. Siebold, 1836.

Syngamus laryngeus Railliet, 1899.
Syn., *Syngamus laryncheus minor* Smit, 1922.
Host. Buffalo, cattle.
Situation. Larynx.
First record of this parasite from Burma.
Family TRICHOSTRONGYLIDAE, Leiper, 1912.
Genus *Haemonchus* Cobb, 1898.

Haemonchus contortus (Rudolphi, 1803).
Syn., *Strongylus contortus* Rudolphi, 1803.
Host. Cattle, sheep, goat.
Situation. Abomasum, small intestine.
Recorded by Bhattacharjee, 1930 [Mitchell, 1931].
Genus *Mecistocirrus* (Railliet and Henry, 1912).

Mecistocirrus digitatus (V. Linstow, 1906).
Syn., *Nematodirus digitatus* Linstow, 1906.
Strongylus digitatus V. Linstow, 1906.
Host. Cattle, buffalo.
Situation. Abomasum.
First record of this parasite from Burma.
Genus *Nematodirus* Ransom, 1907.

Nematodirus filicollis (Rudolphi, 1802).
Syn., *Ascaris filicollis* Rudolphi, 1802.
Host. Cattle.
Situation. Abomasum.
First record of this parasite from Burma.
Genus *Trichostrongylus* Looss, 1905.

Trichostrongylus colubriformis (Giles, 1892).
Syn., *Strongylus colubriformis* Giles, 1892.
Strongylus instabilis Railliet, 1893.
Trichostrongylus instabilis Looss, 1905.
Host. Sheep.
Situation. Abomasum.
Recorded by Giles, 1892 [Bhalerao, 1935].
None in the laboratory collection.
Family *Acuariidae* Seurat, 1913.
Genus *Parabronema* Baylis, 1921.

Parabronema smithi (Cobbold, 1882).
Syn., *Filaria smithii* Cobbold, 1882.
Spiroptera smithi Railliet, Henry and Bauche, 1914.
Host. Elephant.
Situation. Gastric tumour.
First record of this parasite from Burma.
Family GNATHOSTOMIDAE Railliet, 1895.
Genus *Gnathostoma* Owen, 1836.

Gnathostoma spinigerum Owen, 1836.
Host. Cat.
Situation. Stomach.
First record of this parasite from Burma.
Family PHYSALOPTERIDAE Leiper, 1908.
Genus *Chlamydonema* Hegt, 1910.

Chlamydonema praeputiale von Linstow, 1889.
Syn., *Physaloptera praeputialis* Linstow, 1889.
Chlamydonema felineum Hegt, 1910.
Host. Cat.
Situation. Stomach.
First record of this parasite from Burma.
Family SPIRURIDAE Oerley, 1885.
Genus *Spirocerca* Railliet and Henry, 1911.

Spirocerca sanguinolenta (Rudolphi, 1819).
Syn. *Spiroptera sanguinolenta* Rudolphi, 1819.
Host. Dog.
Situation. Oesophagus, stomach, aorta.
Recorded by Bhattacharjee, 1930 [Mitchell, 1931].
Genus *Habronema* Diesing, 1861.

Habronema megastoma (Rudolphi, 1819).
Syn., *Filaria megastoma* Rudolphi, 1819.
Spiroptera megastoma Rudolphi, 1819.
Host. Horse.
Situation. Stomach tumours.
Also recorded by Giles, 1892 [Bhalerao, 1935].

Habronema muscæ (Carter, 1861).
Syn., *Filaria muscæ* Carter, 1861.
Dermofilaria irritans Rivolta, 1884.
Host. Horse.
Situation. Stomach.
First record of this parasite from Burma.
Family THELAZIIDAE Railliet, 1916.
Genus *Thelazia* Bosc, 1819.

Thelazia callipœda Railliet and Henry, 1910.
Host. Dog.
Situation. Eye.
Also recorded by Faust, 1930 [Bhalerao, 1935].

Thelazia lacrymalis (Gurlt, 1831).
Syn., *Filaria lacrymalis* Gurlt, 1831, in part.
Host. Horse.
Situation. Eye. (Aqueous humour).
First record of this parasite from Burma.

Thelazia rhodesi (Desmarest, 1827).
Syn., *Thelazius rhodesii* Desmarest, 1827.
Filaria bovis Baillet, 1858.
Filaria palpebrarum Baillet, 1858.
Host. Cattle, buffalo.
Situation. Eye. (Lacrymal ducts).
First record of this parasite from Burma.

Family FILARIIDAE (Cobbold, 1864).

Genus *Dirofilaria* Railliet and Henry, 1911.

Dirofilaria immitis (Leidy, 1856).
Syn., *Filaria immitis* Leidy, 1856.
Host. Dog.
Situation. Heart, pleural cavity.
Also recorded by Evans and Rennie [1910]. Gaiger [1915].
Bhattacharjee, 1930 [Mitchell, 1931], Bhalerao [1935].

Genus *Onchocerca* Diesing, 1841.

Onchocerca armillata Railliet and Henry, 1909.
Host. Buffalo, cattle.
Situation. Aorta. (In nodules).
First record of this parasite from Burma.

Onchocerca sp. Sweet, 1915.
Host. Cattle, buffalo.
Situation. Subcutaneous nodules.
Recorded by Sweet, 1915 [Bhalerao, 1935]. None in the laboratory collection.

Genus *Setaria* Viborg, 1795.

Setaria digitata (V. Linstow, 1906).
Syn., *Filaria digitata* Linstow, 1906.
Host. Buffalo.
Situation. Eye, peritoneal cavity.
Also recorded by Boulenger [1920]. Thwaite, 1927 [Bhalerao, 1935]. Baylis [1929].

Setaria equina (Abildgaard, 1789).

Host. Horse, cattle.

Situation. Peritoneal cavity, eye, circulatory system, thoracic cavity.

Also recorded by Neumann [1905]. Evans and Rennie [1910]. Bhattacharjee, 1930 [Mitchell, 1931], Bhalerao [1935].

Setaria labiato-papillosa (Alessandrini, 1838).

Syn., *Filaria labiato-papillosa* Alessandrini, 1838.

Host. Cattle.

Situation. Peritoneal and pleural cavities.

Recorded by Bhattacharjee, 1930 [Mitchell, 1931].

Setaria marshalli Boulenger, 1921.

Host. Cattle.

Situation. Peritoneal cavity.

Recorded by Boulenger [1920]. Thwaite, 1927 [Bhalerao, 1935]. Baylis [1929].

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HAEMATOLOGICAL STUDIES IN INDIAN DAIRY ANIMALS

BY

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(Received for publication on 13th February 1937)

(With one text-figure)

Although investigations have been carried out in other countries to fix haematological standards for domestic animals, such work has not been attempted in this country and no records are available of the typical blood picture of Indian dairy animals. Napier and Das Gupta [1935] working on human haematology have found certain deviations in the blood picture of the Bengal population from foreign standards. The present work was undertaken with a view to find out whether any such variation exists between Indian cattle and foreign ones. The buffalo, an important dairy animal in India, is also included. It is hoped that these figures will be of practical value to the veterinarian who has now to depend upon foreign standards.

This paper records the results of a study of the blood of nearly 100 animals of different breeds belonging to the Imperial Dairy Institute at Bangalore. The following were the animals examined, all the cows being in milk :—

6 Working bullocks of Mysore breed.
10 Young Scindi bulls.
30 Half-bred cows.
4 Quarter-bred cows.
1 Five-eighth-bred cow.
1 Three-quarter-bred cow.
1 Ayrshire bull.
1 Three-eighth-bred cow.
20 Scindi cows.
1 Scindi bull.
10 Gir cows.
1 Gir bull.
12 Murrah buffaloes.
1 Murrah buffalo bull.

In addition the blood of four sheep of local breed and two human subjects was examined.

Two or more samples of blood were examined from each animal at different periods. Human subjects were included only to test the correctness of the technique followed. In this work it was possible only to make erythrocyte, leucocyte and differential counts and no attempt was made to estimate haemoglobin, thrombocytes or reticulocytes.

Bleeding and sampling

Samples of blood were drawn either from the jugular vein or the marginal or dorsal vein of the ear. No significant differences between the samples were noticed. At the beginning of these investigations counts were made only on fresh blood. Sometimes owing to accidents the vein had to be punctured again for the second pipette within a few minutes after the first attempt. These latter samples were found to be abnormal due to concentration of leucocytes at this spot. Hence all the counts had to be made on the sample drawn at the very first attempt. This was made possible only by using sodium oxalate [Napier and Das Gupta, 1935] as an anticoagulant, its concentration being one mg. per c.c. of blood.

Blood counts were made on the fresh and oxalated samples to see whether the anti-coagulant interfered with the accuracy of the results. It was noticed (Table I) that the erythrocyte count was unaffected. However, structural changes in the cells were noticed, the red cells becoming crenated and some of the leucocytes failing to take the nuclear stain. Even the film, when stained with Giemsa, appeared reddish, perhaps due to the effect of oxalate on the haemoglobin of the red cells. But in the fresh oxalated samples the lymphocytes alone possessed an affinity for the nuclear stain, and as the other forms of leucocytes, particularly neutrophiles and monocytes were indistinguishable without their nuclear stain, an apparent increase in the lymphocytes was noted when differential counts were made. This might perhaps be the reason for Kerncamp [1933] saying that lymphocytes increase in oxalated blood.

In the smear prepared with a twenty-four hours sample even the lymphocytes fail to take the nuclear stain. Eosinophile nucleii remain colourless even in the fresh oxalated sample, but the cells could be identified by the stained granular cytoplasm.

Hence, for differential counts and for study of structural characters of blood elements, fresh and pure blood alone should be used. But fresh oxalated blood could safely be employed for erythrocyte and leucocyte counts.

TABLE I

Erythrocyte counts in pure and oxalated blood in millions per c.c.

Animals	Pure and fresh blood	Fresh oxalated blood	Oxalated blood 24 hours old	Oxalated blood 48 hours old
Bullocks	8·400	8·608	8·416	7·976
„	8·632	8·696	7·500	..
Cow	9·892	9·680	9·000	8·232
„	9·082	9·105	8·876	..
„	7·230	7·148	7·069	..
Calf	9·340	9·360	9·084	9·020

DILUTING FLUID

For diluting fluid a 3 per cent citrate solution was employed and the results agree with those obtained by the use of Toisson's or Hayem's fluids (Table II).

TABLE II

Blood counts in three different diluting fluids in millions per c.c. of blood

Samples examined	Citrate solution	Toisson's solution	Hayem's solution
Scindi	7·464	7·160	7·324
Bullock I	9·920	10·456	..
Scindi S	7·192	7·000	..
Gir No. 5	9·020	8·984	9·128
Scindi 253	7·632	7·520	..
Cross-bred cow 576	9·024	8·976	..
Cross-bred cow 472	9·336	..	9·256
Cross-bred cow 409	7·032	..	7·056
Gir 24	9·144	..	9·240

The citrate solution, however, is preferable for the following reasons :—

1. It is easily prepared.
2. It keeps a long time.
3. It gives a clear picture of the red cells with no swelling, no shaded centres, no groupings and with the platelets clearly separated.
4. Filling the blood pipettes with the citrate solution and blowing it out are found to be easier.

For leucocyte counts a 0·4 per cent solution of glacial acetic acid tinged with methylene blue was used. For the estimation of erythrocytes, the blood was diluted in Potain pipettes (1-100) and the actual counting was done over Hawksley's improved Neubar counting chamber. Counting of any 100 squares is advised to be essential, but from experience it has been found that counting of 100 squares distributed over the whole field gives a constant and better average than any other form of counting. The same pipettes were used throughout this enquiry eliminating thereby one source of error. Two or more countings were made from the same sample, sometimes from fresh dilutions, at other times from different samples from the same animal and the averages were recorded in Table III.

TABLE III

Blood counts of healthy animals of Bangalore Farm

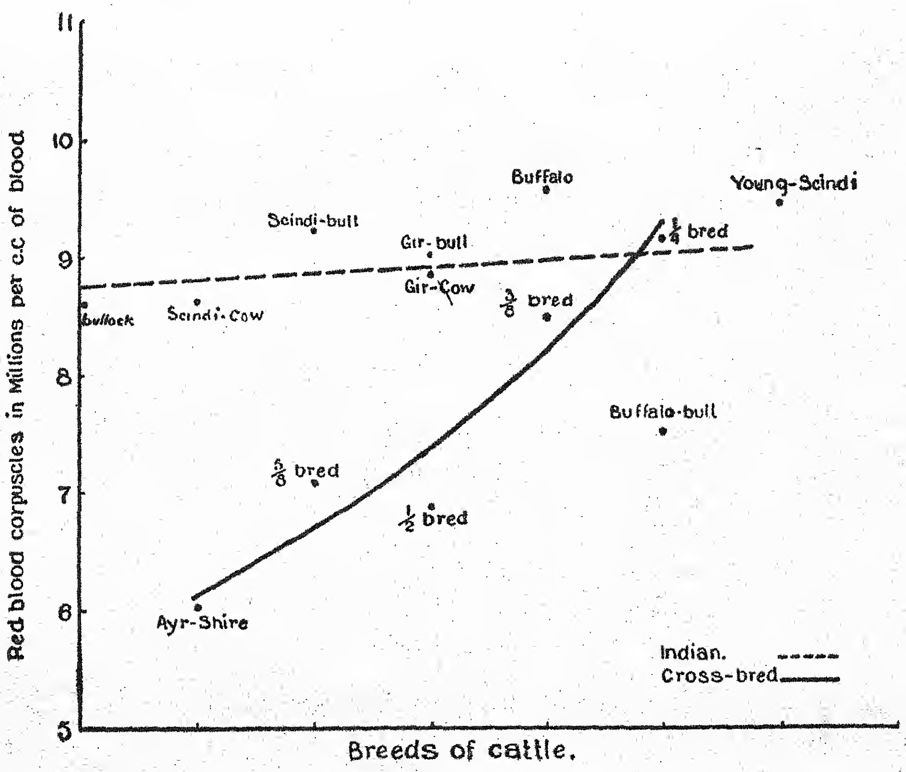
No.	Animals	Erythrocytes in millions			Leucocytes in thousands		
		Maximum	Minimum	Average	Maximum	Minimum	Average
1.	Bullocks (Mysore)	8·732	8·400	8·570	16,000	7,550	12,050
2.	Scindi cows	9·892	7·104	8·498	14,400	12,000	12,840
3.	Scindi bulls	9·554	8·696	9·272	15,200	12,400	..
4.	Gir cows	9·128	6·776	8·923	19,800	10,400	12,000
5.	Gir bulls	..	..	9·024	..	..	15,600
6.	Buffaloes Murrah	11·296	7·544	9·682	20,800	10,400	13,200
7.	Buffalo bull	..	..	7·500	..	..	..
8.	Scindi bulls	9·680	9·072	9·476	..	..	12,000
9.	Half-bred cows	8·044	5·680	6·876	16,000	7,550	11,200
10.	Quarter-bred cows.	9·960	8·592	9·269	16,400	8,600	13,300
11.	Three-eighth-bred cows.	..	..	8·520	..	..	14,000
12.	Five-eighth-bred cows.	7·360	7·672	7·142	..	..	11,600
13.	Ayrshire bull	..	..	6·088	..	..	10,200
14.	Sheep local	14·672	10·280	14·149	12,000	8,200	10,200
15.	Human	5·520	5·376	5·448	..	..	..

Erythrocytes

From Table III, it may be seen that the erythrocyte count in pure Indian cattle is far above those in the Ayrshire bull and the half-bred cows, the mean ranging from 7·5 to 9·6 millions per c.c. of blood, the buffalo topping the list with 9·6 millions average with a maximum count of 11·2 millions. It would be interesting to compare the above figures with those reported by different workers elsewhere. Fraser [1931] working in Cambridge after an extensive study has given " under seven millions " as the average count for British cattle. He further quotes the figures of other European workers which are in close agreement with his own. The American figure of 6·6 millions as reported by Nicols [1935] agrees with that of Fraser. Norris and Chamberlain, quoted by Fraser [1931], working on Australian cattle have reported that the erythrocyte counts range from seven to ten millions per c. c. of blood. However, it is remarkable that the numerical findings for the half-bred cows and the pure Ayrshire bull agree closely with European and American figures and those of Indian cows agree with the figures given for Australian cattle. Friedberger and Frohner [Nicols, 1935] report that there is a tendency to anaemia in dogs of improved and delicate breeds. Napier and Das Gupta [1935] also citing other workers, report that women and men of

India generally give a lower blood count and lower Hb figures compared with Europeans and the reason suggested is that Indians are of lower vitality and stamina. Perhaps the above reasons may also be applicable to cattle.

Another interesting feature seen in the same table is the figure for animals of subsequent crossing. The Bangalore Farm is now experimenting upon quarter-breeding to see whether quarter-bred cows could be made to suit Indian conditions. So far as the blood picture is concerned the quarter-breds are similar to the pure Indian cattle. The three-eighths-bred and the five-eighths-bred cows have given counts midway between those of half-bred and quarter-bred cows and from the following chart it would seem that the erythrocyte count in the cross-bred animals is inversely proportional to the extent of foreign blood content in them.



The graph for the pure Indian cattle represents the average for them excluding the buffaloes. The fact that the bulls give higher counts than the respective cows is also noteworthy, and this fact is also recorded by Fraser [*loc. cit.*]. The buffalo bull, however, gave a lower count and this may be due to the old age of this particular animal.

Leucocytes

From Table II it may be observed that the white cells have a range from seven to twenty thousands per c. c. of blood including the buffalo with an average of 13·4 thousands. Fraser's figure is from three to fifteen thousands for European cattle which also is lower than that of the Indian cattle. Nicols' figure for American cattle is nine thousands which is also low. Nicols [*loc. cit.*] also finds a wide variation in the white cell counts of normal animals amounting to 100 per cent. However, Indian cattle have not given a figure lower than 7,000 and the upper limit is 19·8 thousands in Gir cows and twenty thousands in buffaloes. Such high figures have also been obtained by Sergent and his collaborators [Fraser, 1931] in the case of Algerian cattle. The remarks made about erythrocyte counts in Indian and foreign cattle hold good in leucocyte counts also. But it is remarkable to note that the half-bred cows while agreeing with European cattle in erythrocyte counts differ from both European and Indian cattle in leucocyte counts. The other cross-bred animals show the same relationship to the half-bred cow in their white cell count as in that of the red cell count. Here also the quarter-bred cows behave exactly similar to pure Indian cattle.

As already stated human subjects and sheep were included to test the accuracy of the technique and it is interesting to note that the human figures for erythrocytes closely agree with the standards. In sheep, Fraser has recorded a range from 7·6 millions to 14·4 millions per c. c. for red cells and 4·4 to 13·0 thousands for white cells; whereas the figures for local sheep are 10·2 to 14·6 millions for red cells and eight to twelve thousands for the white cells, which figures do not vary much from those of Fraser except in the range.

Examination of blood smears

The film for studying the erythrocytes and for making differential counts of leucocytes was made by the ordinary method of taking smears direct from the puncture. Of the two stains Giemsa's and Leishman's recommended for staining the film, Giemsa's is found to be more satisfactory for reasons stated by Fraser [*loc. cit.*]. After washing, the film appears light pinkish blue in the case of normal blood. If the smears are made from the oxalated blood, the film appears light brick red as stated already. This same colour has also been noticed to a certain extent in films made from non-oxalated anaemic blood. Thus Giemsa's stain forms a sort of indicator of anaemic blood.

In the stained film the red cell appears pinkish. In all samples examined Microcytes and Megalocytes were observed and with the exception of Gir cattle no animal showed Poikilocytes, Punctate basophilia or any such pathological specimen of red cells. This fact indicates that the animals examined were in normal health so far as the erythrocyte count is concerned. This remark applies to buffaloes also. One noteworthy character in the buffalo blood is that there is a tendency for the red cells to form into rouleaux and the smear is likely to be mistaken for that of the horse but for the differences in the character of the leucocytes.

Among the leucocytes, the small lymphocytes far exceed the number of other forms put together and they are usually found in large numbers at the beginning of the smear, while the polymorpho-nuclears are found mostly at the finish. The appearance of the polymorpho-nuclears at the tail end of the smear may perhaps be due to their lightness or phagocytic movements causing them to be swept off towards the tail end in the act of spreading the drop of blood. The concentration of the different forms of leucocytes in different parts of the film makes it necessary to adopt a special method of counting, so that the apparent and the actual counts may not be far different. Series of fields in lines from one end of the film to the other end when counted, give more or less uniform results.

The following observations were recorded :—

1. In healthy cattle only four kinds of leucocytes were observed and whenever any other form was noticed there was always a variation in erythrocyte, leucocyte or differential counts from the healthy standards.

2. Monocytes are largest in cattle.

3. Polymorpho-nuclears have more well-defined cytoplasm and the neutrophile granules were more prominent in the case of the Ayrshire bull.

4. The polymorpho-nuclears are relatively small and the Eosinophile granules are finer in cows than in the case of buffaloes.

The following is the tabulated record of percentages of different forms of leucocytes found in different breeds. Each figure represents the average of the group. In each film more than 300 cells were counted. In these figures large mono-nuclears and small lymphocytes are grouped as mono-nuclears.

TABLE IV

Differential counts

No.	Animals	Polymor	Eosinoph	Mono.	Range per cent		
					Poly.	Eosino.	Mono.
		Per cent	Per cent	Per cent			
1.	Mysore bullocks	21	9	70	16—22	7—10	69—76
2.	Scindi cows	31	11	57	20—42	8—18	44—70
3.	Scindi bulls	32	16	53	20—42	8—18	44—70
4.	Gir cows	23	8	70	19—28	2—15	44—71
5.	Gir bulls	19	11	71	19—28	2—15	44—71
6.	Buffaloes	28	6	66	21—54	2·5—9	44—73
7.	Buffalo bull	33	9	61	21—54	2·5—9	44—73
8.	Young Scindi bull	20	8	71	18—24	8—9	68—74
9.	Ayrshire bull	26	16	56	..	..	..
10.	Half-bred cows	28	10	61	16—41	6·5—17	47—80
11.	Five-eighths-bred cows	30	13	58	..	..	..
12.	Quarter-bred cows	24	8	67	24—40	5—9	51—66
13.	Average for Indian cattle	26·7	10	64	..	..	..
14.	Fraser's average	30·7	11·2	57·9	16—56	1·2—25	36—80

The figures in Table IV represent the percentage of different kinds of leucocytes present. The standard fixed for foreign cattle by Fraser is also given for comparison. It can be seen that the figures for the Scindis agree well with those of Fraser while those of the cross-bred cows and buffaloes to a certain extent approach them. It may be assumed, therefore, that the percentage of leucocytes is the same in all animals. The only animals which showed variations are the Gir cows and the bullocks. Perhaps this is due to the fact that the Gir cattle were anaemic and the bullocks were under maintenance experiments.

The wide range under each count is difficult to explain. Low neutrophile count, however, is indicative of absence of bacterial disease. High eosinophile count is believed to indicate helminthiasis. Nicols says that the presence of high eosinophile counts, when faecal examinations are negative, indicate that other methods of detecting parasitism should be resorted to [*loc. cit.*]. Napier and Das Gupta [*loc. cit.*], mention that menials in Bengal have less eosinophiles in them than Europeans, contrary to expectations of helminth infection. The buffalo with its filth-loving habits should be expected to have a high helminth infection but gives the lowest eosinophile counts; whereas all the bulls which are under

preferential treatment and are well looked after in the farm give a higher percentage. Again, the Gir cows which are found to be anaemic, which itself may be due to helminthiasis, show a lower count. However, the following points which were noticed are worth recording here :—

1. All mature bulls show a higher eosinophile count.
2. Gir cows give a low count and they are found to be anaemic.
3. Scindi bulls too young for service give low counts.
4. Buffaloes are found to contain less number of eosinophiles. The condition of the animals is a little plethoric.

During the course of this study there was not much chance of studying the blood of sick cattle. However, during a mild outbreak of Foot-and-Mouth disease in the farm, the opportunity was availed of and a few samples of blood were examined and the results are given in Table V.

TABLE V

Blood counts in animals suffering from Foot-and-Mouth disease

Animals	Differential counts in health			Rise of temperature				Fall of temperature				After recovery		
	Poly.	Eosin	Lymp.	R. B. C.	Diff. count			R. B. C.	Diff. count			Diff. counts		
					P.	E.	L.		P.	E.	L.	P.	E.	L.
Cross-bred . . .	30	8	62	...	42	0·5	57·5	...	16	13	70	35	12	56
„ „ 2 . . .	26	11	63	7·0	41	12·0	47	5·21	20	10	70	30	13	57
Scindi cow . . .	30	9	61	9·0	42	0·5	56	6·79	29	6	65	...	...	...
Quarter-bred . . .	25	12	63	9·0	40	9·0	51	7·94	18	9	72	31	8	61

It is seen from the table that during the rise of temperature, there is rise of neutrophiles at the expense of other forms. When the temperature comes to normal a rise in mono-nuclears particularly lymphocytes was observed. After complete recovery the percentages of different white cells are found to have been restored to the pre-fever stage.

Among the different herds examined, some of the Gir cows showed anaemic characters in the structure of the red cells. Further details of the blood picture are given in Table VI.

TABLE VI

Blood counts in some Gir cows of Bangalore farm

Animals	Types	Erythrocytes in millions	Leucocytes in thousands	Differential counts			
				Polymor.	Eosino.	Mono.	Others
Cow 28	Brown .	6·776	19·800	20	16	62	2·0
,, 30	,, .	7·472	11·400	24	2	70	4·0
,, 21	,, .	7·136	10·400	24	15	60	1·0
,, 24	'Kala Boli'	9·144	12·600	22	10	69	1·0
,, 16	,, .	6·552	16·600	40	7	44	7·0
,, 1	,, .	6·440	10·600	25	10	63	2·0

SUMMARY

A study of the blood elements of cattle of the Bangalore Farm was made to fix haematological standards for Indian dairy cattle.

The advantages and disadvantages of using anti-coagulants for collection of blood samples for examinations are discussed. Mention has also been made of the merits of citrate solution as the diluting fluid for the red cell pipette.

Average counts of different breeds of cattle including buffaloes are given in a tabulated form. The erythrocyte and leucocyte counts are found higher in Indian cattle than European cattle, but half-bred cows give figures agreeing with European cattle. Among the Indian cattle the buffaloes give the highest counts, the red cells reaching 11·0 millions and the leucocytes 20·0 thousands.

From a study of the differential counts made on the animals, it is noticed that the different forms of leucocytes exist in the same proportion in all healthy cattle, the proportion being about thirty per cent polymorpho-nuclears, ten per cent of eosinophiles and sixty per cent of mononuclears consisting of both large mononuclears and small lymphocytes. Basophiles are found to be absent in healthy cattle.

ACKNOWLEDGMENTS

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My thanks are also due to Dr. F. J. Warth, the late Physiological Chemist, Bangalore, and Dr. K. C. Sen, Officer-in-charge of the Animal Nutrition Section, Izatnagar, for the encouraging interest taken in this work.

The author wishes to express his gratitude to Mr. Z. R. Kothawalla, the Imperial Dairy Expert, Bangalore, for permitting him to take blood samples from the dairy herd at Bangalore.

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A PRELIMINARY REPORT ON CANINE SCHISTOSOMIASIS IN THE MADRAS PRESIDENCY*

BY

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(With Plate XII)

THE first case of schistosomiasis in a dog in India was recorded by Rao and Ayyar [1935]. The animal was brought to South India from Jubbulpore where apparently, it got the infection in the *jheels*. The subject of this paper is a dog, aged about eighteen months, belonging to a flower merchant of Gudiattam, North Arcot District. This dog, it is reported, was born in Kuppam of Chittoor District, and as a pup, was taken to Gudiattam where it remained most of the time before it was brought to Madras. The owner said that it is very fond of swimming and he allowed it this pleasure in the ponds in his lands at Gudiattam.

The clinical history of the case is that it has had frequent attacks of dysentery and is not keeping such good condition as the owner expects it to have, consistent with the care bestowed upon it. The examination of its faeces showed a large number of ova of schistosomes resembling those of *S. suis* [Rao and Ayyar, 1933].

This is the second case of dysentery in a dog caused by schistosomes to be reported from India and the first indigenous case to be reported in the Madras Presidency. It is possible that a careful search into the causes of dysentery in dogs may reveal more cases of schistosomiasis.

This finding of schistosome ova resembling those of *S. suis* in the faeces of a dog from North Arcot District adds importance to the previous findings referred to below. Rao and Ayyar [1933] found *S. suis* in pigs brought from North Arcot District and slaughtered in Madras. Pigs, when being driven from this district to the various markets, would naturally disseminate infection to certain species of snails in ponds *en route*. It, therefore, seems possible that this dog contracted schistosomiasis in one of such infected ponds in which it was allowed to swim. The author has had no opportunities of examining pigs slaughtered in districts other than North Arcot, and hence is unable to report the existence of this schistosome in other pig-breeding areas of this Presidency. There is some evidence to show that *S. suis* exists in Bengal and Central Provinces. Maplestone collected some from pigs slaughtered in Calcutta and these were described by Bhalerao [1934].

*Paper read at the 24th Session of the Indian Science Congress held at Hyderabad. Deccan, January 1937.

Rao and Ayyar [1935] reported the case of a dog from Jubbulpore which evidently had *S. suis* infection. Hence it may be that this parasite is more widely spread in India than is known at present; nor is there any information regarding the pathological conditions set up by this parasite in pigs and the consequent economic loss to the pig-breeding industry in India.

Description of the ovum. It was possible to obtain ample material for study of the ova. The blood-tinged flakes of mucous contained a very large number of living, dead and ill-developed ova. The dimensions in microns are as follows:—Length 90 to 125. Breadth 40 to 65. Length of spine 5 to 7·5. One side of the ovum is slightly flattened, making it sub-oval in shape and the spine is subterminal, making a wide angle with the flattened side (Plate XII, figs. 1 and 3). In some ova, the spine is curved slightly away from the flattened side or base, though the majority of them have their spines inclined towards it. The flattened side in some of the well-developed ova showed a slight concavity so as to give the ovum a shape somewhat like that of a bean.

Description of the miracidium. The miracidium inside the egg is apparently enclosed in a vitelline membrane which becomes more evident when the ovum is put in fresh water. The miracidia, while in the egg, show up the details of their anatomy through the transparent egg-shell. Many of the miracidia, while still in the egg, appear to possess what may be called 'shoulders' on the antero-lateral margins as depicted in Plate XII, fig. 1 (*c*). Such a structure has not been observed by the author in the miracidia of any of the other species of schistosomes studied by him so far. These 'shoulders' are noticeable just at the places where the posterior set of secretory glands open in the liberated miracidia.

The miracidium, after hatching out of the ovum, appears to spread itself out somewhat, during which process it seems to be stationary. This enlargement is perhaps due to the release of pressure to which it is subjected while in the egg. Very soon it becomes active, swimming away vigorously in water almost in a straight line till deflected by an obstruction in its course.

The miracidium (Plate XII, fig. 2), when killed with five per cent formalin solution, is roughly almond shaped. Anteriorly, it has a nonciliated snout or papilla with an oral opening at its apex. The oral opening leads into a short and blind digestive sac, fully packed with refractile granules. The anterior secretory gland, one on either side of the digestive sac, is rounded, bearing a long duct which opens anteriorly at the base of the snout on each side. These glands are packed with large oxyphilic granules. Each of them together with the duct is about twice as long as the digestive sac. About the middle of the body, in the median line, is situated a rounded compact structure, composed of a number of cells filled with fine basophilic granules. This is the posterior secretory gland from which two ducts, one on each side, appear to arise and each opens on a short papilla in the region of the 'shoulder' referred to above. There is a ring of nonciliated cuticle

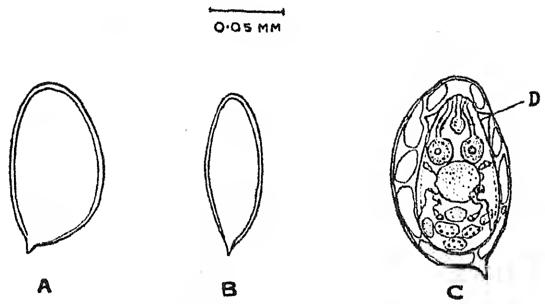

FIG. 1.—Ova of *S. suis* in the faeces of a dog

A. Lateral view of ovum ; B. Dorsal view of ovum ; C. Ovum with Miracidium in it ; D. Shoulder referred to in the text

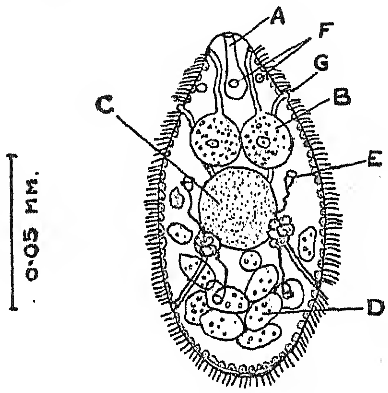

FIG 2.—Miracidium.

A. Gut ; B. Anterior Secretory gland ; C. Posterior Secretory gland ; D. Germinal Cells ; E. Flame cells ; F. Refractile bodies, nature of which is not known ; G. Opening of C

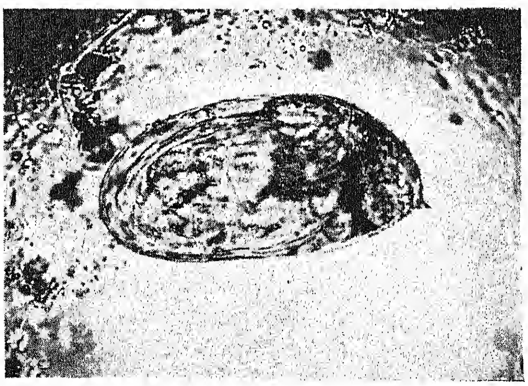

FIG. 3.—Photomicrograph of Ovum

Note the germinal cells inside miracidium

round the body of the miracidium at this region. Another similar ring is seen round the region of the excretory pores in the posterior third of the body. In the body of the miracidium are found a large number of polyhedral germinal cells [Plate XII, figs. 1 (c) and 3]. The cuticle shows an inner lining of rows of tiny cells, the germinal epithelium.

The excretory system consists of a pair of flame cells on either side of the mesial half of the miracidium. The anterior flame cell is situated in a level with the posterior secretory gland and the posterior flame cell is in a level with or a little behind the excretory pore. The tubule from each flame cell unites with its fellow from the opposite direction just behind the posterior secretory gland. The common duct thus formed, after looping to some extent, opens in the excretory pore on the margin of the posterior third of the body.

Some interesting features in the anatomy of this miracidium are the absence of the ciliary girdle and the presence of some refractile bodies, about three in the visual section or six in all, around the digestive sac. These bodies were found to move within certain limits and their nature and function are not known. Such bodies have not been observed hitherto in the miracidia of the other species of schistosomes studied by the author. A comparison, in a tabular form, of the presence and location, etc., of the ciliary girdle on the different miracidia is interesting and the following table gives the necessary details :—

TABLE I

Name of species	Presence or absence of ciliary girdle	No. of groups of cilia in the girdle	Situation	Name of author describing it
S. haematobium	Present .	Not mentioned	Just anterior to excretory pores	Faust quoted by Faust and Meleney, 1924.
Do. .	Do. .	Described as stationary hairs	A little behind 3/5 of body length from the anterior end	Gordon and his collaborators, 1934.
S. mansoni .	Do. .	Do. .	Do. .	Do.
S. spindalis .	Do. .	6 to 8 in the visual section of the miracidium	A little behind the middle of the body	Rao, 1934.
S. nasalis .	Do. .	10 to 12 in the visual section of the miracidium	About the middle of the body	Do.

TABLE I—*contd.*

Name of species	Presence or absence of ciliary girdle	No. of groups of cilia in the girdle	Situation	Name of author describing it
S. japonicum .	Absent .	..	..	Faust and Meleney, 1924.
S. suis . .	Absent .	There appear to be six refractile rounded bodies around the region of the digestive sac.	..	Present author.

From the above table it is seen that the miracidia of *S. japonicum* and of *S. suis* do not possess a ciliary girdle and though, to that extent, there is similarity between the two, yet they are two different species according to Rao and Ayyar [1935].

The dog in question is under treatment with sodium-antimony tartarate and the result is awaited.

SUMMARY

1. An indigenous case of dysentery in a dog due to schistosomiasis in the Madras Presidency is recorded.

2. It is concluded, from a study of the ova, that the infestation is due to *S. suis* [Rao and Ayyar, 1934].

3. The miracidium obtained is described. The interesting points in its morphology are the absence of a ciliary girdle and the presence of six refractile rounded bodies in the region around the digestive sac.

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A PRELIMINARY STUDY OF THE INFLUENCE OF ACCESSORY FACTORS ON THE PRODUCTION OF MILK BY INDIAN GOATS

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(With seven text-figures)

The quantity of milk yielded by an animal in a lactation is not a single entity ; it is the resultant of a complex of forces. It is of very great interest to the practical breeder to know what factors affect the yield and how and to what extent they influence the yield. A knowledge of this will enable him to assess the true economic worth of his animals and to control the various factors, as far as it lies in his power, in such a way as to get the maximum return from his stock. It will also help him to explain the underlying physiological processes.

An attempt is made in the following pages to analyse the available data regarding goats with a view to study the influence of various environmental factors on milking efficiency. Although India possesses a population of forty-nine million goats, goat-breeding is in the hands of illiterate villagers who do not keep records. For accurate records we have to turn to the farms where organised breeding of goats is carried on. Unfortunately such farms are rare and as the work has not been in progress for a long time the data available are not sufficiently voluminous to yield conclusive evidence. The reliability of the conclusions presented below is limited by this fact and they should be considered tentative until confirmed by results from other farms.

The data are obtained from the records of the Government Cattle Breeding Farm, Hissar. These records contain all the details which are usually recorded in the case of cows, *viz.*, dates of birth, dates of calvings, lactation yields, days in milk, days dry, yield at peak, average daily yield, etc., etc. For convenience of manipulation the relevant details are copied on cards ; one card is used for each lactation and the figures are entered in a certain fixed order so as to avoid the labour of copying the headings. These cards are dealt out as required by the particular investigation.

The herd consists of 175 goats ; but in some of these all the information is not complete ; rejecting the latter only 103 animals are available for this study. These 103 animals comprise a total of 234 lactations distributed as follows according to age :

TABLE I

Distribution of lactations according to age

Age in lactation	No. of lactations	Percentage of total
1st lactation	103	43·63
2nd „	62	26·59
3rd „	41	17·61
4th „	20	8·66
5th „	8	3·51
Total	234	100·00

Average age : 2·0085 lactations.

It will be seen from the above that the majority of the goats are very young.

The distribution of these 234 lactations according to the month of kidding is set forth in Table II.

TABLE II

Month of kidding	No. of lactations
January	20
February	24
March	16
April	16
May	11
June	21
July	7
August	29
September	10
October	29
November	19
December	32
Total	234

Though the distribution is not regular the table vaguely indicates that kiddings are more frequent during October to February than March to August.

The distributions of the lactation yields, lactation lengths, service periods and dry periods are given in the form of frequency polygons in Figs. 1 to 4*. The means, standard deviations and coefficients of variations of these characters are given in Table III.

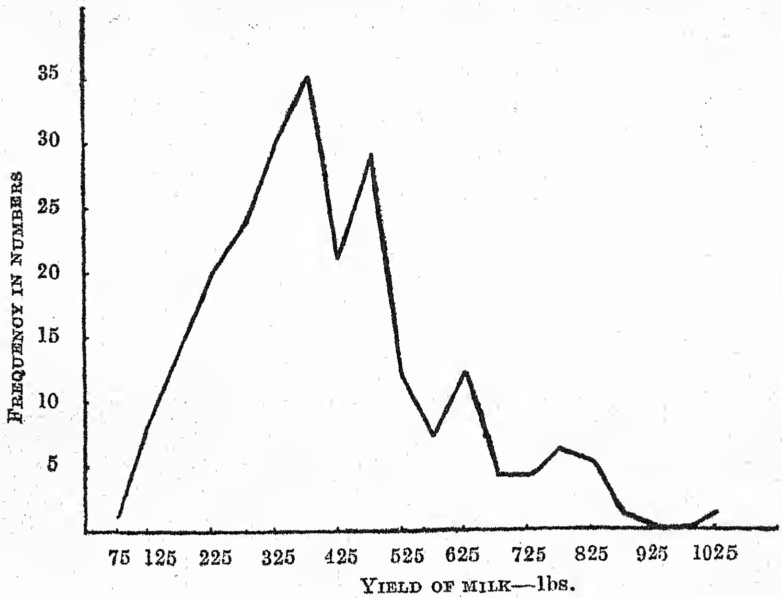

FIG. 1. Frequency distribution of lactation yields

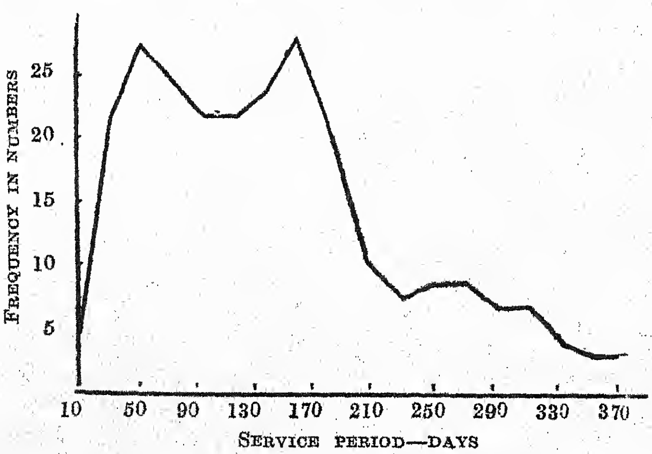

FIG. 2. Frequency distribution of service periods.

* To economise space the tables are not reproduced here. But these can be seen from the correlation Tables IX to XI.

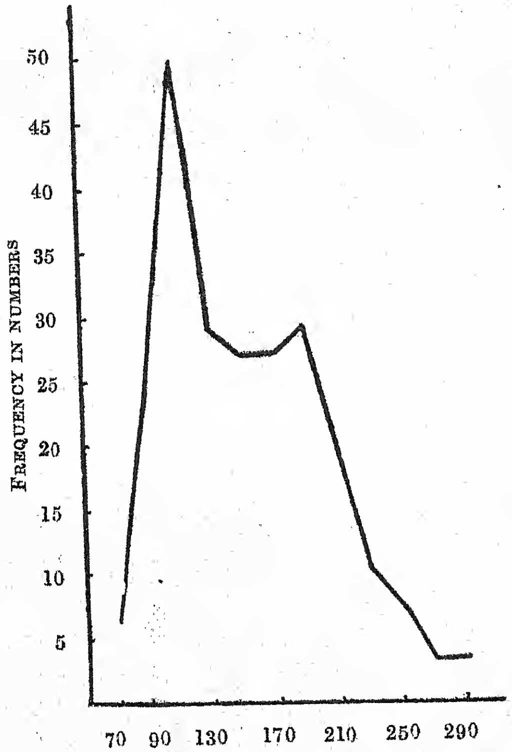

FIG. 3. Frequency distribution of lactation lengths

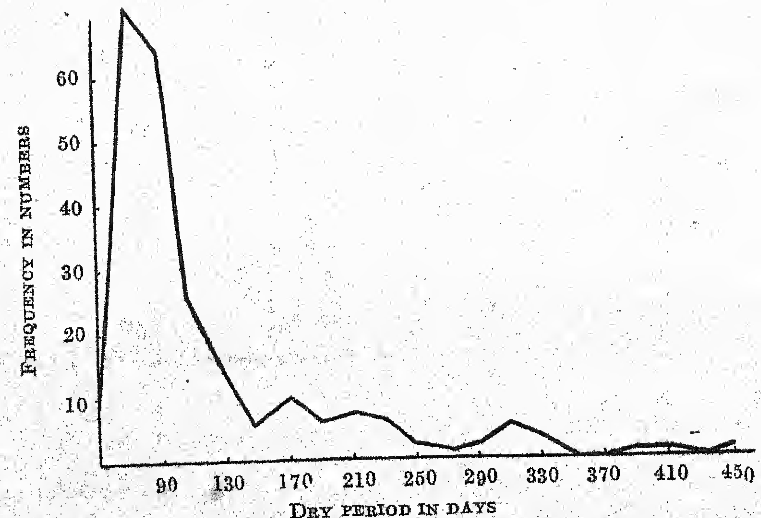

FIG. 4. Frequency distribution of dry periods

TABLE III

Statistical constants of distribution of lactation yield, lactation length, dry period and service period*

Name of constant	Lactation yield lbs.	Lactation length (days)	Service period (days)	Dry period (days)
Mean	403·0	152·64	127·94	119·24
Standard Deviation	171·4	50·64	81·38	74·08
Coefficient of variation . . .	42·53	33·19	63·57	62·16

It will be seen from the coefficients of variation that the 'service' and 'dry periods' are highly variable.

I. THE INFLUENCE OF THE SEASON OF KIDDING

The frequency-distributions of lactation yields, lactation lengths and service periods of lactations commencing in the various months of the year are given in Tables IV to VI and the averages of these characters for the various months are entered in Table VII and illustrated in Fig. 5.

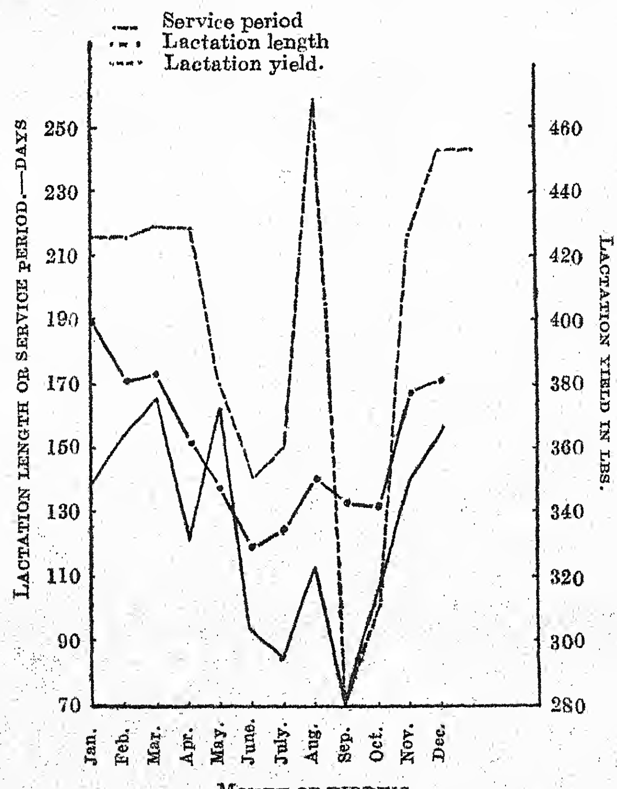

FIG. 5. Influence of season of kidding on lactation length and service period.

* The standard errors of these are not calculated as they are not made use of in this paper.

TABLE IV

The influence of season of kidding on lactation yield

Frequency distribution of yields of milk in lactations commencing in the different months of the year

Yield in lbs. Class mid-points	January	February	March	April	May	June	July	August	September	October	November	December	Total
75	..	..	..	1	..	..	..	..	..	..	..	..	1
125	..	1	1	..	..	1	..	1	1	1	1	1	8
175	..	3	..	..	..	1	2	..	1	2	2	3	14
225	1	1	2	1	..	3	..	1	1	8	1	1	20
275	2	2	..	..	3	3	1	3	2	5	1	2	24
325	4	1	2	3	4	3	1	3	3	4	1	1	30
375	4	2	2	1	2	5	1	6	2	3	3	4	35
425	1	2	3	5	..	2	..	2	..	1	3	2	21
475	3	5	2	3	..	..	..	4	..	3	2	7	29
525	1	..	..	..	..	2	1	2	..	1	..	5	12
575	1	3	1	..	..	..	..	..	..	..	1	1	7
625	2	1	2	1	1	..	..	..	..	1	2	2	12
675	..	2	..	..	1	..	1	..	..	..	..	..	4
725	1	..	..	..	..	..	..	3	..	..	..	..	4
775	..	1	..	..	..	..	..	2	..	..	1	2	6
825	..	..	1	..	..	1	..	2	..	..	1	..	5
875	..	..	..	..	..	..	..	..	..	..	..	1	1
925	..	..	..	..	..	..	..	..	..	..	..	..	..
975	..	..	..	..	..	..	..	..	..	..	..	..	..
1,025	..	..	..	1	..	..	..	..	..	..	..	..	1
Total	20	24	16	16	11	21	7	29	10	29	19	32	234
Mean yield in lbs.	425	425	428·1	428·1	379·5	351·2	360·7	468·1	280	312·9	425	446·9	403

TABLE V

The influence of season of kidding on lactation length

Frequency distribution of lengths of lactations commencing in different months of the year

Lactation length in days.	January	February	March	April	May	June	July	August	September	October	November	December	Total
70	..	..	..	..	..	2	..	1	..	..	..	3	6
90	..	2	1	2	..	4	3	2	2	5	2	1	24
110	4	2	2	..	3	7	1	10	2	12	3	4	50
130	..	2	..	4	3	3	1	6	2	5	1	2	29
150	1	3	2	3	3	3	..	2	3	3	3	1	27
170	7	5	1	4	2	1	1	2	..	..	1	3	27
190	..	3	8	2	..	..	1	2	..	1	3	9	29
210	3	4	1	1	..	1	..	2	1	..	3	3	19
230	..	2	..	..	..	..	..	..	..	1	2	5	10
250	2	1	1	..	..	..	..	2	..	1	..	..	7
270	1	..	..	..	..	..	..	..	..	1	1	..	3
290	2	..	..	..	..	..	..	..	..	..	..	1	3
Total	20	24	16	16	11	21	7	29	10	29	19	32	234
Mean lactation length (days)	188·0	170·0	172·5	151·25	137·3	118·4	124·3	140·4	132·0	131·4	167·9	170·0	152·64

TABLE VI

The influence of season of kidding on service period

Frequency distribution of service periods of lactations commencing in the different months of the year

Service period in days Class mid-point	January	February	March	April	May	June	July	August	September	October	November	December	Total
10	..	..	..	..	..	..	1	..	..	2	..	2	5
30	..	1	..	2	..	4	2	6	1	4	..	1	21
50	5	..	..	2	..	4	1	5	4	5	1	..	27
70	1	3	..	3	3	4	..	2	3	1	2	2	24
90	2	2	..	1	2	4	..	2	..	6	2	..	21
110	2	3	2	2	1	1	1	..	1	..	2	6	21
130	1	6	4	..	2	..	1	2	..	2	2	3	23
150	3	2	2	2	..	..	..	6	1	2	5	4	27
170	2	2	5	..	1	2	..	1	..	1	2	3	19
190	..	..	..	..	..	..	..	2	..	3	..	4	9
210	..	2	1	1	..	..	..	..	..	1	1	..	6
230	1	..	..	1	..	..	1	1	..	..	..	3	7
250	..	..	1	1	..	1	..	1	..	1	1	1	7
270	..	1	..	1	..	..	..	..	..	1	1	1	5
290	2	..	1	..	..	1	..	..	..	..	..	1	5
310	1	..	..	..	..	..	..	1	..	..	..	..	2
330	..	1	..	..	..	..	..	..	..	..	..	..	1
350	..	..	..	..	..	..	..	..	..	..	..	1	1
370	..	..	..	..	..	..	..	..	..	..	..	..	..
390	..	..	..	..	..	..	..	..	..	..	..	..	..
410	..	..	..	..	..	..	..	..	..	..	..	..	..
430	..	..	..	..	2	..	..	..	..	..	..	..	2
450	..	..	..	..	..	..	..	..	..	..	..	..	..
470	..	1	..	..	..	..	..	..	..	..	..	..	1
Total	20	24	16	16	11	21	7	29	10	29	19	32	234
Mean service period—days.	138	152·5	165	121·25	162·7	92·9	84·3	112·1	70	104·5	140·5	155	127·94

TABLE VII

Influence of season of kidding.

Month of kidding	No. of lactations averaged	Average lactation yield	Average days in milk	Average service period	Average daily yield from kidding to kidding
January	20	425	188	138	1·647
February	24	425	170	152·5	1·407
March	16	428·1	172·5	165	1·358
April	16	428·1	151·25	121·25	1·579
May	11	379·5	137·3	162·7	1·214
June	21	351·2	118·4	92·9	1·444
July	7	360·7	124·3	84·3	1·543
August	29	468·1	140·4	112·1	1·786
September	10	280·0	132·0	70·0	1·273
October	29	312·9	131·4	104·5	1·227
November	19	425·0	167·9	140·5	1·461
December	32	446·9	170	155	1·466
Total	234	403	152·64	127·94	1·45

It will be seen from these that kiddings are not distributed over the various months in any regular order, except that in the months of May, July and September they are less frequent. Greatest frequency, however, occurs during the season October to February, showing that the period May to September is more favourable for conception than the remaining period of the year. The larger frequencies in June and August cannot be explained properly; but for these two irregularities the goats may generally be said to be prone to freshening during the winter months.

With the exception of an irregularity in August, the average lactation yields are generally above the average of the herd for November to April and below the average during the remaining six months. But the average lactation lengths and service periods follow the same trend. The goats kidding in May to October conceive quicker than others and consequently go dry sooner. This necessarily makes the lactation lengths shorter and the average lactation yields lower. (The influence of lactation length and service period on lactation yield is examined in a later section). But a lower lactation yield does not mean lower economic efficiency. That animal is economically most efficient which yields the largest quantity of milk during a given period of time, and when lactations are studied the economic worth is best measured by the average daily yield during each

kidding interval. These averages are given in the last column of Table VII. They do not afford any definite indication that kidding in a particular season would enhance the economic value of the animal. On the other hand it would be advantageous, in the interests of a level supply of milk, to have the matings controlled, as far as possible, in such a way that kiddings are evenly distributed in the different months of the year.

Seeing that lactation yields vary according to the season of kidding, it is necessary, in judging animals by their lactation yields, to have a measure of the variation. Figures in Table VIII are worked out with this object in view. These figures indicate what percentage should be added to or subtracted from lactation yields of goats kidding in the various months of the year so as to reduce them to the average for the whole year.

TABLE VIII*

Correction for month of kidding

Month of kidding	Correction per cent
January	—5·18
February	—5·18
March	—5·87
April	—5·87
May	+6·1
June	+14·7
July	+11·7
August	—13·91
September	+43·9
October	+28·7
November	—5·18
December	—9·84

The paucity of numbers in the different months limits the value of these corrections. With the exception, however, of September and October kidders, the correction is not of considerable magnitude.

* See footnote to Table XII.

TABLE IX

Correlation between lactation yield and service period

Days—service period. Class mid-point.

Lactation yield. mid-point / Class	10	30	50	70	90	110	130	150	170	190	210	230	250	270	290	310	330	350	370	390	410	430	450	470	Total
5	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1
125	1	..	1	..	2	..	..	1	1	..	..	..	1	1	..	..	..	..	..	..	..	..	..	..	8
175	2	3	2	1	..	..	2	1	..	1	..	..	1	..	..	..	1	..	..	..	..	..	..	..	14
225	1	4	5	1	2	..	1	..	2	2	..	..	1	1	..	..	..	..	..	..	..	..	..	..	20
275	..	5	4	3	2	3	4	1	1	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	24
325	1	5	2	6	3	..	1	6	1	..	1	1	..	..	2	..	..	..	..	..	..	1	..	..	30
375	..	1	7	4	3	6	1	2	4	1	2	..	2	1	1	..	..	..	..	..	..	..	..	..	35
425	..	1	3	3	4	2	2	1	3	1	..	..	..	..	..	..	..	1	..	..	..	..	..	..	21
475	..	..	2	1	3	3	5	5	3	1	..	2	2	1	..	..	..	..	..	..	..	..	..	1	29
525	..	1	1	2	1	2	..	2	2	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	12
575	..	..	..	..	..	..	4	1	1	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	7
625	..	..	..	1	..	4	..	3	1	1	1	..	..	..	1	..	..	..	..	..	..	..	..	..	12
675	..	..	..	..	..	..	1	..	..	..	1	1	..	..	..	..	..	..	..	..	..	1	..	..	4
725	..	..	..	..	..	..	1	..	..	1	..	..	..	..	..	2	..	..	..	..	..	..	..	..	4
775	..	..	..	2	..	..	..	1	..	1	1	1	..	..	..	..	..	..	..	..	..	..	..	..	6
825	..	..	..	..	1	..	1	2	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	5
875	..	..	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1
925	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
975	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
1,025	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1
Total	5	21	27	24	21	21	23	27	19	9	6	7	7	5	5	2	1	1	..	..	..	2	..	1	234

Mean service period . . . . . . 127·94 days. Standard deviation of service period . . 81·38 days.
Mean lactation yield . . . . . . 403 lbs. Standard deviation of lactation yield 171·4 lbs.
Coefficient of correlation . 0·2658.

Influence of service period

Table IX shows the correlation between service period and lactation yield. The coefficient of correlation is as low as 0·2658 which would seem to indicate that service period has little or no influence on the yield of milk in a lactation. This is contrary to experience. The explanation lies in the fact that the duration of the milking period is dependent not only on pregnancy but on other factors as well—goats seem to go dry early in lactation even though they do not happen to be pregnant. This will be clear from Table X.

It is seen from this table that in quite an appreciable number of cases the service period exceeds the lactation length, showing that at least in these cases it is not the advance of pregnancy that is responsible for the cessation of lactation. The coefficient of correlation between lactation length and service period is only 0·4037. This confirms the fact that pregnancy is not the sole factor that determines the length of milking period. From the records it is not possible to find out which of the animals have gone dry owing to the advance of pregnancy and which for other reasons. The other factors that conceivably influence the length of milking period may be sickness, the plane of nutrition, etc. This requires a separate investigation. The length of lactation has a marked effect on the lactation yield. This will be seen from Table XI.

TABLE X

Correlation between lactation length and service period

Lactation length in days Class mid-point	Class mid-point. Service period—days																								Total
	10	30	50	70	90	110	130	150	170	190	210	230	250	270	290	310	330	350	370	390	410	430	450	470	
70	1	2	..	..	1	..	..	..	..	..	..	..	2	..	..	..	..	..	..	..	..	..	..	..	6
90	3	7	3	3	1	..	1	1	1	1	..	..	1	1	..	..	1	..	..	..	..	..	..	..	24
110	1	9	15	3	3	1	5	2	5	2	..	..	2	2	..	..	..	..	..	..	..	..	..	..	50
130	..	2	7	9	2	1	3	1	1	1	..	1	..	..	1	..	..	..	..	..	..	..	..	..	29
150	..	1	2	7	5	4	1	1	1	..	..	1	1	..	1	..	..	..	..	..	..	1	..	1	27
170	..	..	..	2	6	9	..	2	1	2	2	..	..	1	1	..	..	..	..	..	..	1	..	..	27
190	..	..	..	..	3	6	7	5	2	..	..	2	1	1	..	1	..	1	..	..	..	..	..	..	29
210	..	..	..	..	..	..	5	8	4	..	..	1	..	..	1	..	..	..	..	..	..	..	..	..	19
230	..	..	..	..	..	..	1	4	3	2	..	..	..	..	..	..	..	..	..	..	..	..	..	..	10
250	..	..	..	..	..	..	..	3	1	1	2	..	..	..	..	..	..	..	..	..	..	..	..	..	7
270	..	..	..	..	..	..	..	..	..	..	2	..	..	..	..	1	..	..	..	..	..	..	..	..	3
290	..	..	..	..	..	..	..	..	..	..	..	2	..	..	1	..	..	..	..	..	..	..	..	..	3
Total	5	21	27	24	21	21	23	27	19	9	6	7	7	5	5	2	1	1	..	..	..	2	..	1	234

Mean lactation length . . . . . 152·64 days.
Standard deviation of lactation length . . . 50·64 days.
Mean service period . . . . 127·94 days
Standard deviation of service period . 81·38 days
Coefficient of correlation . . . 0·4037.

TABLE XI

Correlation between lactation yield and lactation length

	Days in milk												
Yield	70	90	110	130	150	170	190	210	230	250	270	290	Total
75	..	1	..	..	..	..	..	..	..	..	..	..	1
125	2	4	2	..	..	..	..	..	..	..	..	..	8
175	3	6	5	..	..	..	..	..	..	..	..	..	14
225	1	2	13	2	2	..	..	..	..	..	..	..	20
275	..	4	11	2	3	2	1	1	..	..	..	..	24
325	..	3	6	7	5	3	4	..	..	1	1	..	30
375	..	3	7	6	8	5	3	3	..	..	..	..	35
425	..	1	3	2	4	4	4	1	2	..	..	..	21
475	..	..	3	3	2	5	7	6	3	..	..	..	29
525	..	..	..	4	1	1	2	2	2	..	..	..	12
575	..	..	..	..	..	..	3	3	..	..	..	1	7
625	..	..	..	..	1	4	1	1	1	3	..	1	12
675	..	..	..	1	..	1	1	..	..	1	..	..	4
725	..	..	..	..	..	..	2	..	..	1	1	..	4
775	..	..	..	1	1	2	..	..	..	..	1	1	6
825	..	..	..	1	..	..	..	2	1	1	..	..	5
875	..	..	..	..	..	..	..	..	1	..	..	..	1
925	..	..	..	..	..	..	..	..	..	..	..	..	..
975	..	..	..	..	..	..	..	..	..	..	..	..	..
1,025	..	..	..	..	..	..	1	..	..	..	..	..	1
Total .	6	24	50	29	27	27	29	19	10	7	3	3	234
Mean of columns .	166·7	237·5	287·0	419·8	388·0	471·3	492·24	511·8	565·0	632·1	608·3	658·3	..

Mean yield . . . . . 403 lbs. Mean days in milk . . . . 152·64 days
Standard deviation of yield . . . 171·4 lbs. Standard deviation of days in milk . 50·64 days
Coefficient of correlation . 0·6448.

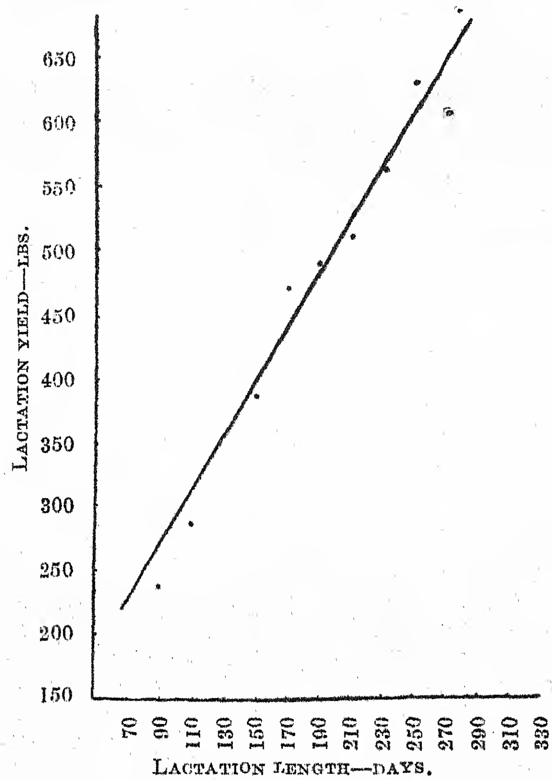

FIG. 6. Relation of lactation yield to lactation length.

The coefficient of correlation is 0·6448. Figure 6 shows the increase in milk yield corresponding to an increase in the length of lactation. It will be seen that the curve cuts through the data fairly well. The regression equation shows that on an average the yield of milk in a lactation increases at the rate of 2·18 lbs. per day increase in the length of lactation. The equation obtained is—

$$Y = 2 \cdot 18x + 70 \cdot 24$$

where Y is the lactation yield in lb. and x the length of milking period in days. There is a fundamental fallacy in assuming a linear relation between the two, for this would show that the yield goes on increasing indefinitely as the lactation is prolonged and that there would be some yield even if the animal does not remain in milk for a single day. But ordinarily such extremes are not met with and corrections based on this equation can be applied within easonable limits of lactation lengths that are found in practice. A better method of obtaining figures of correction would be from the lactation curve based on the knowledge of persistency. But as this is not available the following are given tentatively.

TABLE XII

*Correction for lactation length**

Lactation length in days	Percentage to be added to or subtracted from lactation yield to obtain yield for 150 days
60— 79	78
80— 99	49
100—119	28
120—139	12
140—159	..
160—179	—10
180—199	—18
200—219	—25
220—239	—31
240—259	—35
260—279	—40
280—299	—43

The influence of age and dry period is not examined. As the majority of the goats studied are first kidders no preceding dry periods are available in their case; and until they have remained in the farm for some years sufficient data are not available for studying the influence of age.

* These corrections are based upon raw lactations and not on those once corrected for season of kidding. The working out of accurate corrections for the elimination of the influence of various factors is a complicated matter and should be done by the method of partial correlations. But as this is only a preliminary study and as sufficient material is not available for studying all the factors it is not thought necessary to go into greater detail at this stage. What is intended now is only a very rough indication of the approximate amount of variation caused by individual factors.

When corrections for length of lactation are applied, corrections for season may be ignored; for we have already seen (*vide* Table VIII) that (1) except for September and October kidders influence of season is not considerable and (2) part of the influence of season is due to difference in lengths of lactation.

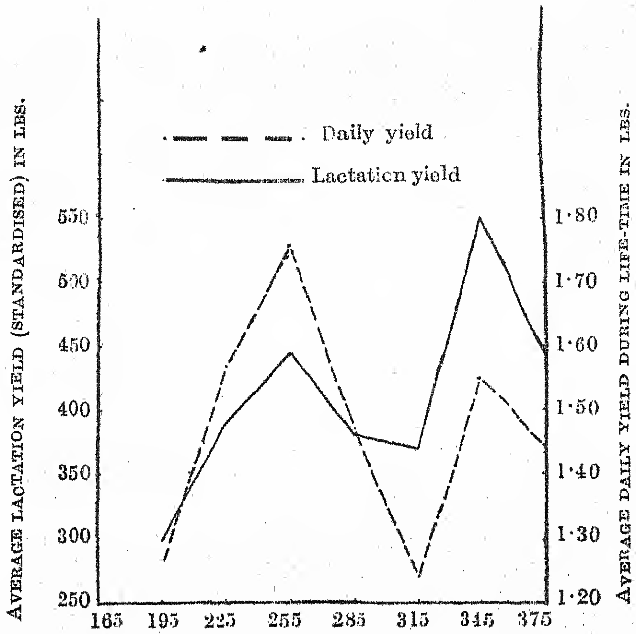

FIG. 7. Relation between average kidding interval and average yield.

OPTIMUM KIDDING INTERVAL

Sufficient material is not available for examining the more important question of optimum length of kidding interval. Rejecting those which have not remained in the herd for at least three lactations we are left with only thirty-five goats. Their average kidding intervals, lactation lengths, daily yield during lifetime, etc., are presented in Table XIII. Two indices are adopted for measuring the economic worth—one is the standardised lactation yield (Standardised to a normal length of 150 days by making use of corrections given in Table XII) and the other the average daily yield during life-time. The kidding intervals are classified into six to seven months, seven to eight months, etc., and averages during these intervals are worked out. These are represented graphically in Fig. 7, the yields being plotted against the mid-point of the intervals represented on the X-axis. It will be seen that the standardised lactation yield as well as the average daily yield during life-time increases gradually with increase in the length of kidding interval, reaches a maximum when the kidding interval is 255 days (*i.e.*, 8 to 9 months) and then falls to a minimum at 315 days. The yield again rises, but the data beyond the interval of 315 days are unreliable as the population on which the average is based, is only one in one case and two in the other case. This leads one to the conclusion, that the maximum return is obtained out of a goat when she is made to kid once every eight or nine months, *i.e.*, by serving her between three and four months after freshening.

TABLE XIII

Average lactation yield and average kidding interval during life time

No.	No. of lactations averaged 1	Total yield of milk during life-time 2	Total days in milk (a) 3	Total days dry (b) 4	Total kidding interval (a+b) 5	Average lactation yield during life-time 6	Average kidding interval during life-time 7	Average lactation length 8	Average day period 9	Average daily yield during life-time 10
1	3	1,473	546	605	1,151	491	384	182	202	1·28
2	4	2,198	637	784	1,421	550	355	159	196	1·55
3	3	886	338	347	685	295	228	113	116	1·29
4	3	1,603	561	303	864	534	288	187	101	1·85
5	5	2,616	840	407	1,247	523	249	168	81	2·10
6	3	804	464	348	812	268	271	155	116	·99
7	5	1,806	532	588	1,120	361	224	106	118	1·61
8	4	2,182	671	494	1,165	546	291	168	124	1·88
9	4	2,033	553	560	1,113	508	278	138	140	1·83
10	4	2,372	727	747	1,474	593	369	182	187	1·61
11	3	892	327	435	762	297	254	109	145	1·17
12	4	1,166	497	303	800	291	200	124	76	1·46
13	4	742	456	317	773	186	193	114	·79	·96
14	3	1,284	468	301	769	428	256	156	100	1·67

15	5	1,729	693	849	1,542	345·8	308	139	170	1·12
16	4	1,716	789	473	1,262	429	316	197	118	1·36
17	5	2,422	715	518	1,233	484	247	143	104	1·96
18	5	1,512	585	719	1,304	302	261	117	144	1·16
19	5	1,628	734	467	1,201	326	240	147	93	1·36
20	4	2,176	870	297	1,167	544	202	218	74	1·86
21	4	1,542	527	474	1,001	385·5	250	132	119	1·54
22	3	1,002	400	323	723	334	241	133	108	1·39
23	5	1,442	778	576	1,354	288	271	156	115	1·06
24	4	1,501	512	503	1,015	398	254	128	126	1·57
25	4	2,663	604	392	996	666	249	151	98	2·68
26	3	1,605	556	184	740	565	247	185	61	2·29
27	3	914	451	362	813	305	271	150	121	1·13
28	3	1,044	423	343	766	348	255	141	114	1·37
29	3	1,118	468	366	834	373	278	156	122	1·35
30	3	1,121	509	315	824	374	275	170	105	1·36
31	3	1,082	439	239	678	361	226	146	80	1·60
32	4	1,043	460	300	760	261	190	115	75	1·37
33	3	1,614	485	247	732	538	244	162	82	2·21
34	3	1,420	485	219	704	473	235	162	73	2·01
35	5	1,880	877	486	1,376	376	273	175	97	1·38

Summary

Data are presented showing the influence of season of kidding and length of lactation on lactation yields of goats ; and corrections are worked out for eliminating the effect of these factors. It is found that excepting in the case of September and October kidders the corrections in respect of season are not of considerable magnitude. Length of lactation has an appreciable effect on the magnitude of the lactation yield.

Goats are found to go dry very often for reasons other than pregnancy. The influence of service period could not, therefore, be ascertained.

Sufficient data are not available for studying the influence of age and dry period.

The limited material available goes to show that the optimum kidding interval for goats is eight to nine months, which corresponds to a service period of three to four months.

The volume of data being very limited, the conclusions drawn are only tentative.

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SELECTED ARTICLE

RECENT DEVELOPMENTS IN THE CHEMISTRY OF VITAMINS*

BY

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GENERAL OBSERVATIONS

THE subject of vitamins is now a very common one. Even a non-scientific man knows about it. These vitamins at one time were considered to be something abstract, whose actual chemical entity was very doubtful and I think I can say that many well-known men of science used to be rather sceptical about the real existence of vitamins. Our requirements are complex. Mere food with nitrogenous substances like protein, etc., will not supply enough to keep us functioning normally. We must have certain things more which we call accessory factors. The so-called accessory food factors which have not been heard of by the preceding generation and which were regarded 25 years ago by men even in scientific circles as elusive and imaginary bodies have now been proved to be definite chemical entities.

It seems that we require some training with regard to our food, otherwise we feed ourselves wrongly and acquire some disease. Without vitamins we suffer from certain diseases. If we take too much of them, we suffer from some other diseases, which are known as 'Hypervitaminosis', one example of this being rickets. If you want the formation of heavy and strong bones you want a lot of vitamin D, but if you take too much of it you get pains all over the body. One of the worst effects of 'Hypervitaminosis' is the calcification of the entities—they become completely calcified and that results in a dreadful disease which was unknown to our forefathers. The isolation of vitamins in the pure state has introduced a new source of danger, namely the opportunity it has given to humanity, particularly faddists, to load themselves with these vitamins and to

* Being extracts from his Readership lectures delivered under the auspices of the University of Madras on 24th November 1936.

suffer from 'Hypervitaminosis', *i.e.*, a surfeit of vitamins, the symptoms of which are worse and more to be dreaded than 'Avitaminosis' or a deficiency of vitamins.

It was known for a long time that there were certain diseases caused by a lack of some nutrient in our food. There is something in the food the absence of which causes us disease. This vitamin deficiency has been traced to certain very common diseases. It sometimes takes the form of beri-beri, which is very common in India, particularly among the populations who use rice as their staple food. Beri-beri is caused by the lack of a certain type of vitamin. Rickets is also caused by the absence of a particular vitamin. We have a common disease known as scurvy which is a skin disease, and which is found to be caused by the absence of the particular vitamin C. There is a curious resemblance of vitamins to the hormones and the enzymes in the physiological activity when occurring in minute traces, but the vitamins differ from the latter, inasmuch as they cannot apparently be synthesized by the body and have to be obtained from some outside source. Thus there is an element of change in the matter of supplying the essential vitamins in our foods which explained the occurrence of such diseases as mentioned above, affecting the human race brought up on highly artificial diets, although the primitive man, with his simple habits and tastes and healthy instincts for natural food, had no difficulty in obtaining all the essential vitamins in his food.

It was Hopkins who first laid the foundation of quantitative nutrition work just before the war, so that really the subject of nutrition is hardly 25 years old. The employment of this method of animal feeding experiment in a strictly quantitative sense has revealed the existence of quite a number of hitherto unrecognised members of the different types of vitamins and led to our present knowledge of the subject. This work has been taken up by other workers in different parts of the world. The same method is adopted in India in the Coonoor Nutrition Institute.

There are a few other workers in India, not very many, carrying nutrition work. Either rats, guinea pigs, or pigeons which are available in large numbers are found suitable animals for experimentation. Striking progress has been made possible only by the international co-operative efforts of teams of workers qualified in such diverse subjects as medicine, physiology, organic chemistry, physical chemistry and even statistics.

The six vitamins regarding the chemical identity of which there remains little or no doubt at present, have been named A, B_1, B_2, C, D and E. I will tell you briefly the sources of each and the method of concentration and also the manner in which they are tested, both chemically and biologically.

Vitamin A is found in all kinds of calorial matter, in fruits and also in milk and butter and in certain oils obtained from fishes like cod, halibut or other sea-fish. It has been found that sea-water-fish liver contains a fairly large amount

of vitamin A. The vegetable sources are plantains, bananas, tomatoes, carrots, spinach, apricots, etc. Apricots are considered recently by American workers to be the best and the richest source of vitamin A.

In a concentrated form vitamin A is prepared chiefly from cod-liver oil. Here we have national patriotism. An amusing example of this is afforded by the rival claims of Professors Drummond and Hilditch who, on behalf of the British Empire Marketing Board, had emphasised the unrivalled medicinal value of the cod-liver oil produced in Scotland and New-foundland, whilst the Norwegian workers claimed their cod-liver oil to be superior and to possess twice the potency of the New-foundland oil. However, it is gratifying to learn that the Indian fish-liver oils particularly that from "Vetki", a fish quite common in the Madras market, are much superior to the cod-liver oil in this respect.

An Indian chemist, Guha of Calcutta, published results of his work on vitamin A, by the expedient of measuring the intensity of the blue colour developed by mixing the liver oils with antimony trichloride in chloroform solution under standard conditions. I wish you can find out the concentration of vitamin A in the particular substance. The method that he used was very simple and can be carried out by any person without any training. The liver of the fish was taken out, thoroughly ground with sodium sulphate or hydro-sodium sulphate and this powdered dried substance was weighed and extracted rapidly with ether. It dissolved in antimony. The substance was dried and it was spontaneously evaporated under an electric fan leaving a deep yellow oil. He took 4 c.c. of oil and dissolved it in 4 c.c. of chloroform and treated this with the solution of anti mony, left for 30 seconds and in this way he obtained the following results :—

Vetki (Lates Calcaufer)	284	Carr. No. units.
Rohit (Labeo Rohita)	227	Do.
Mrigae (Cirrhive Mrigala)	174	Do.
Katla (Cutla)	109	Do.
Hilsha (Chigia Itisha)	59	Do.
Cod	5	Do.
Halibut	614	Do.

(*Jour. of Med., Chir. Sec.*, 1933—P. 364-365).

I have been asked by a worker in Bengal whether the ghee which we make by clarification of butter does contain the whole of the vitamin which was present in the butter in spite of our having boiled it at high temperature on the oven for the preparation of ghee, but this gentleman was of opinion that the vitamin present in the butter is lost in the preparation of ghee from butter. Of course, I do not know how far his experiment is reliable, but it is interesting to know more about it, particularly because it concerns all of us, who depend upon ghee as a very important constituent of our food.

Prolonged vitamin A deficiency produces diseases of the nervous system chiefly of the mucous membranes and lastly, by no means the least, it causes the person who is suffering from this deficiency to be readily attacked by infectious diseases.

Vitamin B is always found on the outer surface chiefly in seeds, cereals, grains, pulses, etc. It is found in the embryo of the seed and not on the whole of the seed. In the case of rice it is the main bulk which we eat, but the embryo is a very small part and this embryo is simply lost during the milling and polishing operations. If you want polished or milled rice, it is mostly deprived of the embryos and it is vitamin-free and there lies the danger of populations depending on this rice as their food, suffering from deficiency diseases caused by the lack of vitamin B_1, chiefly beri-beri. Vitamin B_1 is found in eggs, particularly in the embryo tissues, also in fish livers, fruits, chiefly figs, grapes and lastly it is found in that wonderful substance called " yeast ". Yeast is now the chief source of this vitamin. Vitamin B_1 has been prepared in a pure condition from yeast. There have been schools of workers on vitamin B_1 throughout the world. All these workers prepared almost simultaneously crystalline B_1 hydrochloride. It was really wonderful how each arrived at the same result almost at the same time.

The biological assay of vitamin B_1 deficiency was established recently, by the electro-cardiographic method of Harris of determining the heart beats of rats suffering from vitamin B_1 deficiency and the careful study of the conditions under which they develop. This method has proved invaluable in identifying the crystalline vitamin B_1 hydrochloride, synthesized by Williams a few months ago with the natural vitamins, the method of measuring the resistance of the capillary walls of the skin in vitamin C deficiency by observing the minimum pressure required to produce haemorrhages in the skin under standard conditions, the calcification or " lime test " of McCollum, based on X-ray examination of the bones of puppies, etc. Although these appear to be rather complex and difficult to carry out, the experimental technique has been perfected to such an extent in individual laboratories as to make these well-defined quantitative tests of great accuracy serve to detect and estimate the nearest traces of particular vitamin occurring in different food-stuffs.

The most interesting fact we have recently concerning vitamin B_2, the growth-promoting factor, is its identification with certain organic compounds which are called flavines. These are nitrogenous organic matter which have been found to be characterised by the yellow colour. I may tell you that ghee-yellow contains flavine. It will be surprising to know how ghee contains flavine. It does. Flavine prepared from various sources were tested and they all agreed in one characteristic, namely the yellow colour. Ghee-white has been found to give vitamin B_2.

Vitamin C has been found chiefly in fruits, among which are oranges, grapes, lemons. Seeds contain none, unless they germinate. Eggs likewise contain none. There is a special variety of vegetable in Hungary known technically as 'Vitapric'. This is the richest source of vitamin C.

The application of the spectroscope in the study of vitamins has met with phenomenal success, most of the vitamins being found to give very characteristic absorption bands in the ultra-violet region of the spectrum, the intensity and persistence are found to correspond quantitatively to the amounts of vitamins present.

II. Structural formulae of the vitamins A and B

Although the vitamin problem might justly be regarded as bio-chemical in its inception, the interest had widened considerably in the course of the last three years and it would be more appropriate now to treat them as problems of outstanding interest for the organic chemist.

With regard to vitamin A, two distinct lines of attack had been most fruitful of results, *viz.*, the examination of the oils derived from the livers of fish, chiefly cod and halibut, and the exhaustive study of certain vegetable pigments named "Carotenoids" or Lipochromes, which had been extracted from such common vegetables as carrots, tomatoes, maize, etc. While the concentration of the unsaponifiable matter of the liver oils constituting nearly one per cent of the original oil and containing all the vitamin activity, by crystallisation, precipitation of impurities like sterols and fractional distillation in a high vacuum led, on the one hand, to the isolation of a fairly pure specimen of this vitamin, the intensive study of the pigment of carrots resulted on the other in the isolation of an orange red crystalline substance which was found to have the action of vitamin A when fed to animals. Although this red colouring matter, which had been named "Carotene" and the purest form of vitamin A were similar in their biological action and also in many of their chemical properties such as destruction by oxidation and ozonisation and the development of a blue colour with antimony trichloride, the recognition that they were different bodies was not long to follow, the discrepancies being finally explained by the brilliant work of Moore, who during the years 1929-31, demonstrated conclusively that carotene was converted into vitamin A in the animal body ; it thus became clear that carotene was an immediate precursor of vitamin A and that plants could synthesise only carotene but not vitamin A while the animal organisms converted the carotene into vitamin A chiefly in the liver. The experimental work on the carotenoids and of Karrer's preparation of pure vitamin A from halibut liver oil have opened a new method of purification of compounds of these types which had been named "chromatographic analysis" and which had proved to be of the greatest value in these researches. The method, technique of which had been perfected by Kuhn, Zechmeister and others, was shown to be, in principle, essentially that of capillary

analysis, being based on the fact that the adsorbability of a number of allied substances on any one adsorbent is frequently susceptible of very fine differentiation enabling a separation between them being made which was impossible by other methods. This chromatographic adsorption analysis is now the most delicate method at the disposal of the chemist for work in this field, the purification of vitamin A by Karrer and also by Heilbron and others having been accomplished with its aid.

" Perhydro-vitamin A " was synthesised in the laboratory of Karrer in Zürich and this could be identified with that obtained from the natural vitamin by reduction. (This part of the lecture was illustrated fully with lantern slides).

The original vitamin B_1 curative to beri-beri, variously known as "Aneurin", " Torulin ", " Oryzanian ", etc., has been the subject of much research by Jansen and Van Veen in Java, Ohdake in Japan, Windaus in Germany, Peters in England and Williams in America, who had all prepared in their laboratories the pure vitamin in crystalline form at about the same time hardly a year ago. A rough idea of the difficulties of experimental work with this vitamin which had been prepared by diverse workers from rice, bran, rice polishings, yeast, etc., would be realised from the figures given recently by Peters in a paper wherein he showed that 4½ tons of yeast had to be worked up to get about 3 grms. of this vitamin under the most favourable conditions. The previous work of Windaus made us understand the oxidation products of this vitamin and how the precipitation properties and the adsorption curves of the vitamin resembled closely those of histidine and cytosine respectively. Further careful investigations by Williams and his co-workers of the Department of Physiological Chemistry, Teacher's College, Columbia University, New York, during the last year culminated in the synthesis of vitamin B_1 hydrochloride three months ago. (The various degradation products of the vitamin and the syntheses were illustrated with slides).

Biologically evidence is growing that vitamin B_1 is concerned in some oxidative mechanism in carbohydrate metabolism, an attractive hypothesis being that it played the role of a co-enzyme in an oxidative enzyme system, *e.g.*, a dehydrogenase. Highly interesting work on these lines is carried out by Peters and his collaborators of the Oxford School who had succeeded in demonstrating the accumulation of abnormal amounts of lactic acid and pyruvic acid in the brains of pigeons suffering from polyneuritis (deprivation of vitamin B_1) and they correlate them with the lowered oxygen consumption of the brain tissues of these birds.

Future years would reveal many other important aspects of the action of these vitamins and I hope, now that their structures have been determined, that chemists would be able to make them available to medicine and biology.

III. VITAMIN B_2

While synthesis had satisfactorily settled the identity of vitamin B_1, vitamin B_2 appeared to be made up of at least two major components which produced different physiological effects, one having the power to prevent or cure the cessation of growth while the other affected the peculiar disease known as pellagra. While these two factors occur associated together in the natural vitamin B_2 concentrate and are difficult to separate, the important discovery has been made in the course of the last three years that the growth-promoting factor of vitamin B_2 belonged to a class of yellow-colouring matters called "flavins," a number of which has been isolated from different sources, *e.g.*, egg-white (ovoflavin), whey (lactoflavin), liver (hepatoflavin), kidneys (renoflavin), and even urine (uroflavin), which were all very similar in properties, displaying a strong yellowish green flourescence in aqueous solutions and being generally stable towards mineral acids and oxidising agents but decomposed by alkalis and by irradiation. The crystalline orange-brown lactoflavin obtained by Kuhn from whey (nearly 1 grm. from 5,400 litres) which represented the most active preparation of the growth-promoting factor of vitamin B_2 had been intensively studied during the last year leading to important developments taking place in the chemistry of these flavins. The investigation of the products obtained by the irradiation of lactoflavin in alkaline and alcoholic solution was followed by the recognition of these bodies as alloxazine derivatives and the establishment of their constitutional formulae by syntheses. From these decompositions a complete picture of the structure of lactoflavin was deduced, a pentose chain being considered on chemical grounds to be attached to the position 9 in the alloxazine ring.

The immediate outcome of these researches was the identification of the natural lactoflavin with 6, 7-dimethyl-9, *d*-ribo-flavin by the exhaustive study of the chemical and biological properties of all the eight possible stereo-isomeric pentose-flavins which had been synthesised. The excellent yields of these products obtained by Kuhn and his collaborators by their processes of synthesis and the strong growth-promoting actions of both the 6, 7-dimethyl and the 7-methyl-9, *d*-ribo-alloxazines encourage the hope that these would be available in quantity for medical practice in the near future. The interesting discovery of the new yellow-oxidation enzyme in yeast juice by Warburg and Christian has now been proved to be made up of lactoflavin phosphoric ester associated with a protein, the growth-promoting factor of vitamin B_2, thus becoming the prosthetic group in Warburg's oxidation enzyme. It is highly important to know this relationship for a proper understanding of the physiology of vitamin B_2 action, inasmuch as it appears to indicate that this vitamin is needed for the elaboration of an essential enzyme concerned with biological oxidations, which the animal organism by itself is incapable of synthesizing.

With regard to the other factor of the "B_2 complex", namely, the anti-pellagra or the anti-dermatitis component, very little is known as yet about its chemical

composition except that it is found to be equally susceptible to inactivation by visible light. This component is now being designated as vitamin B_6, the old name vitamin B_2 being reserved for the growth-promoting factor alone. As a result of recent experiments on the distribution of vitamin B_6, it has been found to be present in considerable amounts in fish muscle (herring, salmon and haddock) and attempts are now being made to prepare it in a pure condition from these sources.

Vitamin C

The determination of the structure of vitamin C and ultimate synthesis constituted another outstanding chemical triumph in recent years. One of the characteristics of vitamin C is that of curing a very well-known disease known as scurvy. This disease is chiefly found among soldiers, prisoners and sailors, particularly among soldiers who are actually in a camp and who mostly depend on tinned rations, prisoners who are often given somewhat starch food materials and sailors who also depend on the food materials which they carry in the vessel. The peculiar characteristic of this disease is the degeneration of the capillary walls of the skin. The generous supply of orange and lemon juice and of fresh vegetables has been known to cure this disease.

It has been recognised that the anti-scorbutic principle is concentrated in fruit-juices. The chemical investigation of vitamin C began only four years ago when several workers in different parts of the world independently and almost simultaneously recognised that the sugar acid isolated in 1928 by the Hungarian investigator Prof. A. Szent-Gyorgyi from the adrenal cortex of oxen which he called 'Hexuronic acid' and the anti-scorbutic principle known as vitamin C were really one and the same. The progress made from this time onwards in the chemical examination of this material has been amazingly rapid, beginning with the discovery of methods of easy preparation of the substance in quantity from a number of sources such as orange and lemon juice and particularly from Paprica or Hungarian red pepper fruits, and ending with the determination of its chemical constitution by Haworth, Hirst and others in co-operation with Szent-Gyorgyi in England, followed finally by the syntheses of the vitamin nearly three years ago by the two schools of research, one led by Haworth in England and the other by Reichstein in Switzerland. Vitamin C has now been named by general agreement "Ascorbic acid" and the contents of this acid of some fruit preparations are given below :—

Ascorbic acid contents of some fruit preparations mg. per grm.

Vitapric	4·5
Paprica (whole fruit)	1·8
Lemon juice	1·78
Spinach flower	1·16
Tomato	1·09
Guava (whole fruit)	1·04

Striking and unexpected effects of vitamin C have been revealed recently by clinical medical experiments. It seems that with the aid of this vitamin an effective cure could be obtained of many diseases such as nephritis, haemophilia, pyorrhoea, etc., against which medicine and surgery were previously helpless. These curative effects in pathological conditions which were never suspected before to have any connection with a lack of vitamin seem to be very suggestive indicating humanity to be suffering probably from a lack of vitamin C on a much more extensive scale than has hitherto been supposed. It is, therefore, considered a great triumph for chemical science that in the short space of a couple of years this mysterious accessory factor of foods had been identified, its structure determined, its synthesis effected and finally made available for medicine and industry.

The recent observations of Prof. Szent-Gyorgyi and others seem to show that in paprica, at any rate, vitamin C is accompanied by a substance of similar importance and related activity, particularly effective in curing certain acute pathological conditions characterised by a morbid fragility of the capillary walls of the skin, which by careful fractionation and study, has been identified as a pure flavon or flavonol-glucoside. These results lack confirmation yet, but if found to be correct, the large group of vegetable dyes known as flavons and flavonols will also have to be added to the list of substances playing an important role in animal life.

IV. Vitamin D

Although the problem of the chemistry of vitamin D had been attacked only some seven years ago, the special deficiency disease known as rickets for which this vitamin was the specific cure, had been recognised much earlier, the condition being principally one of weakness and malformation of the bony structures as might be seen by the deformity of the leg bones and better by means of X-rays, which revealed a curious coarse structure and frayed appearance at the ends of the bones indicative of unsatisfactory calcification. Rapid advances in the study of rickets were made at the time of the Great War when, on account of various circumstances and particularly of the blockade of Central Europe, these deficiency diseases, particularly rickets among children, became very common in those countries. The scientific study of rickets was first made possible by Mellanby in England who experimented with puppies, and showed how this disease could be produced in the puppies at will, certain dietary factors present in cod-liver oil and to a lesser extent in butter being found to play the primary part in curing this disease. The confusion which arose from the similarity of distribution of this anti-rachitic factor for dogs and the growth-factor known as vitamin A was cleared up by the experiments of the American workers, Sherman, McCollum and others, who made the interesting discovery that when the cod-liver oil was heated in air at 100 degrees, the growth-promoting factor 'A' was destroyed, while the anti-rachitic factor remained. Vitamins A, B and C having already been recognised, the new vitamin was called Vitamin D.

A new and rather disturbing factor was introduced into the scientific study of human rickets in 1919, with the discovery that the disease could be cured in children by exposing them to ultra-violet radiation and the realisation which came with it that many hitherto unexplained observations on human rickets, such as the seasonal increase in this disease in late winter and early spring after deficient sunlight, its high incidence in Northern countries with less sun-light and in slum children in smoky cities, could be best correlated with this discovery of the curative effect of sun-light. There were thus two mysterious indeterminates in the picture, namely, the vitamin D of cod-liver oil and ultra-violet radiation, and this led to various speculations, one of the most attractive and popular theories being that ultra-violet radiation synthesized this vitamin in the body of the animal. The right trial was blazed at last by the experiments of Hess, Steenbock and others in America who made the important observation that food freed from vitamin D could be endowed with anti-rachitic properties by irradiation. The progress of the search for the particular constituent of the food which was activatable in this way, resulting finally in the discovery that it was the natural sterols cholesterol in animals and phytosterol in vegetables—associated with the fatty parts of the food, was one of the fascinating romances of modern bio-chemistry.

With this identification of vitamin D with the irradiation product of a sterol, vitamin D entered the stage of a chemical problem and the general features of the molecular structure of sterols characterized by a system of four reduced carbon rings (cyclopenteno-phenanthrene) with one or more unsaturated linkages and an alcoholic hydroxylic group. The examination of the various natural sterols and their activation by sun-light, leading to the discovery of a particular member of this class found in fungi known as 'ergosterol' which was activated more powerfully on exposure to light than any other material which had so far been tested, marked the next important stage in these studies, ergosterol being identified with 'pro-vitamin D'. From this time onwards three groups of workers in England, Germany and Holland competed for the honour of isolating the vitamin itself, the purified vitamin obtained in a crystalline condition and having practically the same physical constants and anti-rachitic activity being announced by them at about the same time. (The methods by which the different preparations were made by the English, the Dutch and the German schools, and the separation from the distilled crystalline material of a pure alcohol which was named "calciferol", having the highest biological activity of any thing previously tested by fractional crystallisation of the 3 : 5 dinitrobenzoic ester, by Bourdillon and his co-workers in the National Institute of Medical Research at Hampstead, London, were then described.)

Irradiation of ergosterol

The next advance was made when it was realised that the irradiation of ergosterol produced a series of photo-isomerides named Lumisterol, Tachysterol,

Calciferol and Suprasterols I and II, which formed stages in a continuous transformation, and which could be isolated by the careful adjustment of experimental conditions and studies. With the collection of this material the chemistry of vitamin D in this sense of structural organic chemistry had just begun to unfold. (The methods which had been particularly serviceable in the elucidation of the structures of these photo-isomerides, such as oxonolysis, catalytic hydrogenation, selenium dehydrogenation and addition with maleic anhydride (Diels-Alder reaction) were discussed in detail and how the constitution of 'Calciferol' had been established with tolerable certainty by these methods was explained with the aid of slides). In the light of these findings, the view which prevailed at the beginning, that the active anti-rachitic principle in fish-liver oils was nothing else than activated ergosterol, and that all foods and animal tissues which were capable of being rendered anti-rachitic by irradiation contained only ergosterol, was shown to be no longer tenable, the accumulated evidence indicating the existence of many other pro-vitamins although the exact nature of these remained unknown. That calciferol or as it might be called the synthetic vitamin D, was different from the natural vitamin D of cod-liver oil, had been demonstrated satisfactorily by several workers recently and it was now considered probable that any sterol which contained a conjugated system of ethylenic linkages in positions 5, 6, 7 and 8 should be capable of being transformed into an anti-rachitic factor by irradiation with ultra-violet light, a view which received striking support lately from an observation made with a synthetic derivative of cholesterol (7 dehydrocholesterol) which could be converted into a highly active anti-rachitic product.

THE NEW ANTI-STERILITY VITAMIN

We shall now consider vitamin E (Evans) which is prepared from the oil extracted from wheat embryos by the usual process of removal of saponificable matter, chromatographic absorption, distillation in high vacuum, etc. This vitamin has been called anti-sterility vitamin. The chemical examination of the most active fraction (a pale yellow oil) obtained in this way showed it to have an unsaturated structure and to be of the nature of a cyclic ketone capable of exhibiting ketonal changes, with a molecular weight of about 450 and having two oxygen atoms one of which could be acetylated. It was fairly stable and could be hydrogenated in the presence of palladium without losing its activity but bromination, acetylation and oxidation destroyed its potency. Although the successful treatment of sterility in cows and of habitual abortion in women by means of this concentrate had been reported recently, the actual function of vitamin E in the body was not definitely known, and the important questions as to whether this vitamin was related in any way to the numerous hormones which regulated the normal reproductive cycle or if the deficiency of this vitamin was associated in any way with disturbances in the functions of the pituitary glands or of the corpus luteum had not yet been adequately examined.

In conclusion, I hope I have made it clear in the discourses which I have been giving during the past week that a great body of accurate knowledge about food and nutrition has been gained recently through the co-operative labours of chemists and biologists. What only a few years ago were mysterious and intangible growth-promoting, anti-scorbutic or anti-rachitic properties in good food-stuffs caused by certain unidentified and probably unidentifiable bodies called vitamin and given vague descriptive letters like A, B, C, etc., had now been proved by the chemist to be definite organic substances the structural formulae of which had been determined and which, in at least three instances, had been synthesized in the laboratory. The consequences of these discoveries could not fail to be of far-reaching importance. Nutrition and food are the first weapons of preventive medicine and the clinician of the future would be enabled to prescribe an exact dose consisting of a given number of International Units of the standardised synthetic vitamins for curing or preventing disease and place the science of nutrition, which was hardly a science upto now, on a scientific and logical basis. Nutrition should be regarded as one of the most important subjects in the present day. It is possible for instance by treatment of population with these vitamins not only to cure existing diseases but also to protect them from the ravages of dreadful diseases like haemophilia, pyorrhoea, nephritis, or some of the diseases like scurvy, rickets and so on.

ABSTRACTS

The Chemotherapy of *Dirofilaria immitis*. HERBERT G. JOHNSTONE. (*Amer. J. Trop. Med.*, **16**, 207-224, 1936).

STUDIES on the chemotherapy of *Dirofilaria immitis* were undertaken by Johnstone with the hope of eventually finding some specific remedy for the treatment of the various types of human filariasis. In an interesting résumé the author points out that various drugs have been extensively used over a period of years for the treatment of human filariasis with disappointing results. Numerous chemotherapeutic agents have been advocated, and upon administration have shown promise during the initial trials only to be discarded following a more widespread application.

No satisfactory treatment for heartworm was known until a group of Japanese workers reported some success with certain antimony compounds. Itagaki and Makin [1927] used intravenous injections of sodium antimony tartarate which caused the disappearance of microfilariæ from the peripheral blood of seventeen dogs. However, living adult worms were found after death of the dogs tested. Wada [1927] treated seven dogs with the average dose of 0·011 grm. of neostibnal per kg. administered subcutaneously for ten to twenty times. Microfilariæ disappeared from the peripheral circulation in all the dogs treated and in one of the animals a dead worm was found. Intravenous injections of carbon tetrachloride and solutions of neutroflavine have also been tried with negative microfilaricidal action.

Philipp [1931] was the first to report good results with Fouadin, a proprietary antimony compound, in the treatment of heartworm disease. Underwood and Wright [1932] reported favourably on the use of Fouadin in four infested dogs. They found that best results were obtained when treatment was pushed to the limit of tolerance in order to obtain a maximum concentration of the drug as soon as possible. Popesco [1933] treated fourteen dogs with Fouadin with excellent results. The first injection varied from 2—5 c.c., and the course varied from four to eight injections over a period of five to fourteen days with a total of 10·5—43·5 c.c. There was a reduction in the number of microfilariæ but no reference to the effect of the drug on the adult worms was made. In an important paper Wright and Underwood [1934] reported that Fouadin is comparatively safe to use if certain restrictions are observed resulting in the disappearance of the microfilariæ and the eventual destruction of some or all of the adult worms in the heart and pulmonary artery. The drug is contra-indicated in cases of organic heart disease or pathologic conditions of liver or kidney. Destruction of a large number of worms all at once may cause embolic pneumonia or an acute toxemia.

Recently Cheu and Khaw [1935] claimed that " Concentrated Fouadin ", a solution containing eleven per cent sodium-antimony III pyrocatechin-disulphonate of sodium and calcium, was four or five times therapeutically more potent in its effects than

Fouadin used by Underwood and Wright [1932]. The author carried out chemotherapeutic experiments to test the effect of large and even toxic doses of Fouadin on the heartworm of the dog. He came to the conclusion that whereas the drug given intramuscularly within a short period of time had marked microfilaricidal action, its large and even fatal doses exerted little or no damaging effects on the adult worms. He also tried the effects of Carbarsone, Hexylresorcinal, Trypanblue or sodium antimonite on the heartworm disease of the dog with negative filaricidal or microfilaricidal results. The effect of Roentgen therapy was also tried on *D. immitis in situ* with disappointing results.

A useful list of forty references concludes the paper. [H. D. S.]

Turning sickness, a protozoan encephalitis of cattle in Uganda. Its relation with East Coast Fever. R. W. M. METTAM AND J. CARMICHAEL. (*Parasitology* **28**, 254, 1936).

" TURNING SICKNESS " or " Vizengerara ", as natives call it, has been reported to cause damage to cattle in different parts of Uganda. In acute cases onset is sudden and animals otherwise known to be healthy develop violent nervous symptoms, such as spinning or turning in one direction with lack of coordination of movement, until dizzy, when they collapse to the ground to rise up again within a few minutes. At this stage the animal is comatose while salivation may be profuse. Muscular twitches at the time of paroxysms are prominent. Respiration is hurried while the pulse is very weak or quite imperceptible. The eyelids are wide open with injected conjunctiva and dilated pupils. Sometimes local hyperaesthesia is also noticeable. A noteworthy feature of the disease is the absence of any fever. At times complete blindness may ensue which may be permanent or temporary. Diarrhœa has not been recorded, though in some cases there was obstinate constipation. In chronic cases the animal appears unthrifty or poor in condition ; generally quite blind in one or both eyes. Turning movements are never severe, violent or continuous. Muscular incoordination and sometimes atrophy of certain muscle groups has been reported. These protracted cases die of exhaustion and death may be quite unexpected. Pathologically, in acute cases, petechial hæmorrhages or extravasations are noticed in various parts of the brain and meninges. Microscopic examination reveals that the brain lesions are caused by the extensive embolism of cerebral vessels and the lymphocytes, which form these emboli, show in their cytoplasm protozoan bodies which are indistinguishable in structure from the schizonts of the genus *Theileria*. In two cases only out of twenty-four, Koch's bodies were seen in the lymphocytes of the peripheral blood. In chronic cases hæmorrhages are absorbed but brain is permanently damaged by replacement changes and by the appearance of small cysts or cavities containing citron-coloured fluid. The disease has not yet been transmitted experimentally. Its connection with Theileriasis is still uncertain but much evidence is on record to show that cases of turning sickness have been infected with East Coast fever sometime previously. [H. N. R.]

The differential diagnosis of equine abortion with special reference to a hitherto undescribed form of epizootic abortion of mares. W. W. DIMOCK AND P. R. EDWARDS. (*The Cornell Veterinarian* **26**, 231-240, 1936).

THE article deals principally with what the authors designate as virus or epizootic abortion. Incidentally, the authors review the history of equine abortion in the U.S.A., and record brief notes on other forms of equine abortion such as salmonella abortion, streptococcic abortion, and abortions due to other miscellaneous causes.

In occurrence and characteristics, the virus abortion is rather typical of an epizootic disease. It often assumes serious proportions.

The diagnosis of this disease must often be approached indirectly through a process of elimination. If the fetuses in an outbreak show the lesions described below, no bacteria are found in the organs and tissues, and the blood of the mares that abort is negative to the agglutination test for salmonella infection, then the disease is probably epizootic or virus abortion.

The affected mares show no premonitary symptoms of approaching abortion. With few exceptions, the afterbirths are passed immediately, and the mares, particularly those aborting between the seventh and ninth month of gestation, do not suffer from any ill-effects. They return to normal as promptly as do mares following normal parturition.

Some or all of the following changes are usually found in the aborted fetuses : An accumulation of serous fluid in the thoracic cavity in over one-third of the cases, particularly in those thrown between the eighth and tenth month of gestation ; liver enlarged, engorged and of a dark-bluish colour ; numerous small white degenerated areas placed just beneath the capsule of the liver ; congestion of the mesenteric and colic lymph glands ; and haemorrhages in the heart in the form of petechial and lineal areas. Occasionally, the first few fetuses from an epizootic may not show the above lesions.

Various attempts were made to implant the virus in small animals and to transmit the disease to pregnant guinea-pigs and pregnant mares. A mixture of a wide variety of material was used to overcome any possible localisation of the virus in one or more of the fetal tissues. Mice inoculated intra-cerebrally with a view to demonstrate a neurotropic virus remained healthy.

Non-pregnant rabbits injected intra-venously, intra-peritoneally and intra-cerebrally and non-pregnant pigs injected intra-peritoneally also remained healthy. Of seven pregnant guinea-pigs, one aborted three immature fetuses and five gave birth to litters containing both live and dead ones. Nine pregnant mares were fed and injected. Two of these aborted, the fetuses presenting the characteristic tissue changes and remaining bacteriologically sterile.

To prove the filterable nature of the etiological agent, the authors infected three pregnant guinea-pigs and ten pregnant mares with filtrates of fetal fluids and organs. All the three guinea-pigs gave birth to dead or immature pigs. Three of the mares aborted, the other seven foaling normally.

Housing in stalls vacated by an aborted mare and feeding with water from the same source as the infected mares did not lead to abortion in pregnant mares. But a mare given the same feed and water as the infected mares and placed in a stall where a mare had aborted and into which fetal membranes of aborted fetuses were thrown and left for two or three hours on each of four occasions aborted.

Although all their experimental cases did not give positive results, the authors consider that abortion was produced in a sufficient number to indicate that the disease is infectious and that the infectious agent is a filterable virus.

The authors recommend the following measures for the control of epizootic abortion :—isolation, maintenance of brood mares in small groups and the subcutaneous or intravenous inoculation of 50 to 100 c.c. of convalescent serum into all incontact pregnant mares. [V. R. R.]

NOTICE

Applications are invited for the "Maynard Ganga Ram Prize" of the value of Rs. 3,000/- which will be awarded for a discovery, or an invention, or a new practical method tending to increase agricultural production in the Punjab on a paying basis. The prize is open to all, irrespective of caste, creed or nationality and Government servants are also eligible for it. Essays and theses are not eligible for competition and applicants should prove that some part of their discovery, invention, etc., is the result of work done after the prize was founded in 1925. The Managing Committee reserves to itself the right of withholding or postponing the prize, if no satisfactory achievement is reported to it. All entries in competition for the next award should reach the Director of Agriculture, Punjab, Lahore, on or before the 31st December, 1938.

ORIGINAL ARTICLES

STUDIES ON THE MINERAL REQUIREMENTS OF CATTLE IN NORTH-EAST INDIA

(WITH SPECIAL REFERENCE TO RICE STRAW FEEDING)

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INTRODUCTORY

The importance of minerals in the economy of nutrition requires no special emphasis. In ordinary feeding practices, the difference in the protein-content and energy values of feeds may be insignificant, yet due to a difference in the mineral-content some will exhibit definite superiority over others. Thus, Hall and Russell [1912] when investigating the fattening and non-fattening pastures of Romney marsh were unable by ordinary chemical analysis to discover sufficient chemical difference in the protein and starch-equivalent values of the two pastures to account for the great difference in the feeding values obtained in practice [Linton, 1927]. The work carried out at the Rowett Institute by Godden [1926] has shown that the difference in the feeding values of various pastures is to be found not in the content of protein but in the amount of CaO and P_2O_5. A somewhat similar work in Bengal shows that *aman* (winter rice) straw and *aus* (autumn rice) straw have practically the same energy value, but there is a great difference in their mineral-content. It has been found that, even when fed with concentrates, *aus*-straw-fed animals have always yielded better results. An intensive study has, therefore been attempted of some of these aspects under the conditions prevailing in Bengal which may be said to be typical of North-East India.

* Under the grant of the Imperial Council of Agricultural Research.

It is necessary to give here a brief outline of the nature of the feeds used. In North-East India paddy or rice straw is the chief staple fodder. Apart from the variations arising from soil character, the straw can be broadly divided into three kinds : (1) *Aman* or winter straw, (2) *Aus* or autumn straw and (3) *Boro* or spring straw. The first grows in low-lying or submerged areas, the second in high lands and the third near streams and rivers. The whole paddy area in Bengal is nearly twenty-three million acres of which *aman* occupies of 16,442,400 acres or 71·81 per cent, *aus* 6,035,600 acres or 26·34 per cent and *boro* 415,100 acres or 1·71 per cent*. The present paper deals with the mineral aspects of the first two and particularly of the type grown in the red, acid tracts as represented by the Dacca Farm.

The analysis† of the Farm soil gives about 0·1 per cent total and 0·04 per cent citric acid soluble CaO, 0·06 per cent total and 0·005 per cent citric acid soluble P_2O_5 and about 0·075 per cent total nitrogen. The organic matter as represented by loss of ignition is about 3·5 to 4·25 per cent.

The data of materials relating to this paper are incorporated in the relevant parts while the total analysis will be found in Appendices I & II. The more notable points are briefly stated as follows :—*Aman* straw (which is a crop of low water-logged area) is a coarse dry fodder, varying in its nitrogen-content according to the period of harvesting and nature of storage. It is somewhat low in lime, richer in potash and poor in phosphate and nitrogen.

Aus straw is cultivated in high lands and appears to be better than *aman* straw in lime, phosphate, magnesia and nitrogen. It has further the additional advantage of being harvested at a time when it is still slightly green. As already stated the feeding tests with this straw have been followed with better results than in the case of *aman* straw, whether when fed alone or in combination with cake.

Linseed-cake is a well-known concentrate rich in protein and phosphate but with a low lime-phosphate ratio.

Rice *kura* (local name) or rice polishing is available everywhere in the country. It is rather unusually rich in phosphate (over 6 per cent in the samples used), and fat (over 20 per cent). It is fairly rich in magnesia (about 2·6 per cent) and has a protein-content of about half of that of linseed-cake. It is, however, very poor in lime, in fact the poorest amongst the feeds tested (about 0·2 per cent). On this account its CaO : P_2O_5 ratio was about 1 : 30. In spite of its intrinsic richness in some of its important minerals (P_2O_5 and MgO) and possibly of its better biological protein values as noticed by various workers [Boas Fixen, 1935] its unbalanced mineral-content appears to be very large. Guinea grass (*Panicum maximum*) is a departmentally recommended green fodder rich in lime, soda, phosphate and nitrogen.

* Season and Crop Reports of Bengal, 1931-32.

† Unpublished data of Agricultural Chemist's Laboratory, Bengal, 1917.

Napier grass (*Pennisetum purpureum*) is another recommended departmental fodder which is a prolific grower yielding about double the outturn of Guinea grass, but is poor in CaO, rich in potash and also in P_2O_5.

Water-hyacinth can hardly be classed as a fodder but due to fodder scarcity, it is often used and even purchased for feeding cattle in some of the eastern districts. It is rich in lime, potash and chlorine but the poorest in P_2O_5 among the green feeds tested.

Experimental scheme

This has been drawn up so as to conform as far as possible to the nature and type of feeding as often used in the countryside. Thus rice straw is fed in some cases as an exclusive feed, in others in combination. In the experiments, combinations were made with rice polishings and also with linseed-cake, while some with green fodders have also been included.

In these calculations, the mineral requirements have been assumed to bear a direct relation to body-weight instead of its power function as adopted for energy and nutrients of organic origin. The published records so far available to the authors appear to be in favour of this procedure. If, however, subsequent investigators show that power function is more appropriate, necessary alterations can easily be made on these data.

In order to make an easier comparison all the results have been calculated on a fixed live-weight of 500 lb., which is the average on this side of India. Thus, on one side the data may be available for more direct practical application, while on the other, by simply doubling the values, they can be made comparable with the conventional procedure of 1000-lb. live-weight.

The chemical analyses have been mainly conducted by the A.O.A.C. method with slight modifications here and there.

The stall arrangements and procedure of metabolic tests were broadly similar to what are ordinarily followed elsewhere.

The following is a brief outline of the experimental scheme.

Experiment A.—(*aman* straw as a single feed) :—

Low CaO, low P_2O_5 and low protein.

Two animals were used. They were fed straw *ad lib* and the feeding lasted for a period of about eighteen weeks. On the basis of 500-lb. live-weights, the animals received per day about 14 grms. CaO, 5 grms. P_2O_5, 50 to 70 grms. K_2O and 16 to 17 grms. nitrogen, equivalent to about 100 grms. or $3\frac{1}{2}$ ounce of protein.

Experiment B.—*Aman* straw and rice *kura* (bran), low CaO, very high P_2O_5, fairly high MgO, and moderate protein.

Nine animals were used in this experiment which lasted for eighteen weeks. In the mixed feed with straw the ratio $CaO : P_2O_5$ varied between 0·24 to 0·47 or from one-fourth to one-half, *i.e.*, P_2O_5 was about two to four times more than CaO. The straw contributed the largest share of CaO, the share of *kura* being about one-seventh only of that of straw. The ingestion of P_2O_5 was from 40 to 60 grms., the *kura* contributing as much as 13 to 20 times that of straw. The protein may be said to be fairly adequate for maintenance. The nutritive ratio varied from 1 : 24 to 1 : 34.

Experiment C—Aman straw and linseed cake; CaO still low, though higher than experiments A and B; MgO also low, though higher than in experiment A; but P_2O_5, protein and energy supply adequate.

In this experiment four animals D_1, D_3, D_4 and D_7 were used and it lasted for twelve weeks. The nutritive ratio was about 1 : 17 to 1 : 19 except in D_4 at 1 : 22. The nitrogen and energy supply being adequate, the behaviour of minerals and their deficiencies were expected to be related to the amounts present.

Experiment D—Aus straw as a single feed: CaO higher than in experiments: A, B and C, but still slightly deficient, low P_2O_5 and bare protein.

This straw as already stated is richer than *aman* straw in minerals and protein. The CaO ingestions varied from 21 to 23 grms. per 500-lb. live-weight, while P_2O_5 was slightly above that of experiment A, being still a low figure (about 6 grms.) The $CaO : P_2O_5$ ratio was 3·64 and $K_2O : Na_2O$ ratio about 5. The protein supply as indicated from the nitrogen balance was just on the border line. The nutritive ratio was 1 : 23 and the experiment lasted for forty-two days.

Three animals D_1, D_3 and D_7 were used for the test.

Experiment E—Aus straw with half and three-fourth lb. linseed cake: adequate in mineral, protein and energy supply—in short apparently adequate in all the required nutrients.

Like experiment D which was carried out at the same time, it lasted for forty-two days and has the same source of roughage (*aus* straw), but while in the former the straw was an exclusive feed, here it was supplemented with linseed cake. Altogether six animals were used, three under ½ lb. cake and three under ¾ lb. cake but since the computations on the basis of 500-lb. live-weight exhibit only a negligible difference they have been grouped together. The nutritive ratio varied from 1 : 11 to 1 : 14, the $CaO : P_2O_5$ ratio varied from 2 to 2.46 while $K_2O : Na_2O$ ratio was much the same as in experiment B. Its general adequacy in all nutrients provides suitable lines of comparison towards indicating the possible nature of requirements.

Experiment F—Aman straw in combination with green feeds. This experiment is really divided into three parts *viz.*,

(1) Hyacinth group,
(2) Napier group, and
(3) Guinea grass group.

In each, two animals were used. The straw was fed *ad lib* in the morning and green feed *ad lib* in the evening. The water-hyacinth is very rich in CaO (3·42 per cent), MgO (0·96 pe· cent), potash (5·9 per cent) and Cl_2 (3·48 per cent). Napier grass is poor in lime (0·46 per cent) like *aman* straw, but rich in potash (4·8 per cent). Guinea grass is rich in lime (1·23 per cent) and fairly so in magnesia (0·73 per cent). Its soda content (0 57 per cent) was the highest of all other feeds.

This experiment was carried out on the same lines as Experiment A and lasted for about the same period.

Experimental Results

Experiment A.—Low calcium, low phosphate and low protein. This experiment is a typical instance of how in Bengal an exclusive feed of straw upsets the condition of the animals. Of the two animals, D_7 lost 63 lb. during the course of eighteen weeks while D_8 lost 68 lb. The chief nutrients—nitrogen, lime and phosphate—were voided more through the faeces than were supplied in the feed. The main data are set out in Table I.

TABLE I

Aman *paddy straw as a single feed*

EXPERIMENT A *(Computed on 500-lb. live-weight)*

Animal No.	Nutrients	Intake: Straw grms.	Intake: Added grms.	Intake: Total grms.	In faeces grms.	Difference grms.	In urine grms.	Balance grms.	Loss or gain in live-weight lb.
D_7	CaO	14·707	...	14·707	16·510	—1·803	0·383	—2·186	
	MgO	10·636	...	10·636	4·764	5·872	3·792	+2·080	
	K_2O	57·765	...	57·765	11·864	45·901	66·130	—20·229	—3·88
	Na_2O	12·984	10·822	23·806	5·378	18·428	7·305	—11·123	
	P_2O_5	5·098	...	5·098	8·538	—3·440	0·079	—3·519	
	Cl_2	7·115	11·927	19·042	1·627	17·415	19·956	—2·541	
	N	16·981	...	16·981	22·643	—5·662	8·318	—13·980	
D_8	CaO	13·473	...	13·473	17·673	—4·200	0·224	—4·424	
	MgO	9·740	...	19·743	0·428	9·315	1·691	+7·624	
	K_2O	52·919	...	52·919	38·303	14·616	55·015	—40·399	
	Na_2O	11·894	11·081	22·975	6·449	16·526	5·803	+10·723	—3·43
	P_2O_5	4·670	...	4·670	6·927	—2·257	0·079	—2·336	
	Cl_2	6·518	12·212	18·730	1·194	17·536	13·496	—4·040	
	N	15·557	...	15·557	21·137	—5·580	6·770	—12·350	

As will be noted, there was an imbalance as much from protein deficiency as from mineral, in consequence of which the food requirements fell far short of the barest necessity. The dry matter consumption was similar as in the other experiments with better-balanced feeds but here the utilization was very poor.

Experiment B.—Aman straw and Rice bran (*Kura*). Low calcium, very high P_2O_5, fairly high MgO, and moderate protein.

During the eighteen weeks over which the experiment was spread, three animals *viz.*, D_1, D_2 and D_3 maintained more or less the same live-weight but the other six lost from 9 to 37 lb. weight. The straw consumption fell from 20 to 50 per cent between the first and last week. No relation can be traced with the rate of consumption of rice *kura* and the live-weight.

As regards the supply of protein, seven recorded a definite positive balance. Of the two recording a negative balance, D_8 lost an appreciable amount, but this animal was particularly unthrifty from the very beginning. Leaving out these two, the others would indicate that the protein supply was adequate for maintenance. The energy supply was also fairly adequate, yet the animals generally lost in weight and were definitely out of condition. The tendency can hardly be ascribed to an insufficiency of energy supply or of protein or to a lower biological value of the latter as rice protein is credited with a high biological value. The nature of the mineral supplies provides an explanation. These have been set up in Table II.

EXPERIMENT B.

TABLE II

Aman Straw and *Kura* (1934)

(Computed on the basis of 500-lb. live-weight)

Animal No.	Nutrients	Intake: Straw	Intake: *Kura*	Intake: Water	Intake: Added minerals	Intake: Total	In faeces	Difference	In urine	Balance	Daily loss or gain in live-weight
		grms.	grms.	grms.	grms.	grms.	grms.	grms.	grms.	grms.	lb.
D_1	CaO	16·491	1·355	0·128	0·050	18·024	17·755	0·269	0·089	+0·180	+0·59
	MgO	12·027	17·357	0·073	0·059	29·516	22·964	6·552	1·827	+4·725	
	K_2O	62·018	12·790	...	0·142	74·950	18·873	56·077	51·109	+4·968	
	Na_2O	9·200	1·295	...	6·504	16·999	8·945	8·054	7·827	+0·227	
	P_2O_5	3·364	41·082	...	...	44·446	42·773	1·673	0·187	+1·486	
	Cl_2	11·074	0·122	...	7·713	18·909	3·459	15·450	17·037	—1·587	
	N	17·058	16·793	...	...	33·851	22·618	11·233	9·071	+2·162	
D_5	CaO	16·894	1·166	0·116	0·051	18·227	18·955	—0·728	0·079	—0·807	—0·21
	MgO	12·323	14·938	0·065	0·061	27·387	20·905	6·482	1·748	+4·734	
	K_2O	63·535	11·007	...	0·146	74·688	13·153	61·535	58·089	+3·446	
	Na_2O	9·422	1·114	...	6·674	17·210	13·358	4·852	3·087	+1·765	
	P_2O_5	3·489	35·354	...	...	38·843	39·422	—0·579	0·079	—0·658	
	Cl_2	11·345	0·105	...	7·914	19·364	2·808	16·556	18·601	—2·045	
	N	17·688	14·451	...	...	32·139	22·943	9·196	8·565	+0·631	
D_7	CaO	13·696	1·557	0·089	0·052	15·394	16·482	—1·088	0·046	—1·134	—0·34
	MgO	9·991	20·001	0·051	0·061	30·104	23·902	6·202	2·120	+6·082	
	K_2O	51·501	14·737	...	0·146	66·384	18·386	47·998	46·630	+1·368	
	Na_2O	7·636	1·492	...	6·711	15·839	13·668	2·171	2·716	—0·545	
	P_2O_5	2·833	47·336	...	...	50·169	48·396	1·773	0·102	+1·671	
	Cl_2	9·196	0·141	...	7·959	17·296	1·446	15·852	19·106	—3·254	
	N	14·255	19·349	...	...	33·604	23·227	10·377	9·418	+0·959	

TABLE II—*contd.*

Animal No.	Nutrients	Intake: Straw	Intake: *Kura*	Intake: Water	Intake: Added minerals	Intake: Total	In faeces	Difference	In urine	Balance	Daily loss or gain in live-weight
		grms.	grms.	grms.	grms.	grms.	grms.	grms.	grms.	grms.	ib.
D_3	CaO	16·123	1·172	0·109	0·054	17·458	18·828	—1·370	0·068	—1·438	—0·11
	MgO	11·758	15·039	0·062	0·065	26·924	23·906	3·018	3·720	—0·702	
	K_2O	60·635	11·081	...	0·152	71·868	16·592	55·276	51·232	+4·044	
	Na_2O	8·995	1·122	...	6·986	17·102	9·561	7·541	7·229	+0·312	
	P_2O_5	3·320	35·586	...	...	38·906	39·238	—0·332	0·104	—0·436	
	Cl_2	10·828	0·105	...	8·285	19·218	2·098	17·120	19·538	—2·418	
	N	16·808	14·548	...	...	31·356	23·521	7·835	8·245	—0·410	
D_4	CaO	15·237	1·397	0·116	0·045	16·795	19·134	—2·339	0·109	—2·448	+0·44
	MgO	11·111	17·883	0·065	0·053	29·112	20·556	8·556	0·015	+8·541	
	K_2O	57·296	13·176	...	0·127	70·599	16·895	53·704	51·167	+2·537	
	Na_2O	8·497	1·334	...	4·227	14·058	11·348	2·710	1·763	+0·947	
	P_2O_5	3·145	42·320	...	...	45·465	44·714	0·751	0·109	+0·642	
	Cl_2	12·524	0·154	...	6·931	19·609	3·508	16·101	21·659	—5·558	
	N	15·646	17·300	...	...	32·946	22·165	10·781	9·243	+1·538	
D_2	CaO	11·897	2·172	0·091	0·047	14·207	14·402	—0·195	0·432	—0·237	—0·23
	MgO	8·692	23·427	0·052	0·056	32·227	23·325	8·902	4·436	+4·466	
	K_2O	43·752	15·991	...	0·133	59·876	18·795	41·081	46·325	—5·244	
	Na_2O	6·213	4·197	...	6·115	16·525	13·940	2·585	3·462	+0·123	
	P_2O_5	2·573	55·709	...	...	58·282	43·974	14·308	0·128	+14·180	
	Cl_2	9·664	0·264	...	7·251	17·179	1·182	15·997	18·488	—2·491	
	N	14·282	17·252	...	...	31·534	19·936	11·598	11·230	+0·367	

D_3	CaO	14·049	1·901	0·092	0·048	16·090	17·518	—1·428	0·097	—1·525	
	MgO	10·255	20·440	0·053	0·057	30·805	22·764	8·041	3·618	+4·423	
	K_2O	51·646	13·988	...	0·137	65·771	15·616	50·155	53·204	—3·049	
	Na_2O	7·333	3·662	...	6·298	17·293	9·974	7·319	10·739	—3·420	+0·83
	P_2O_5	3·037	48·618	...	...	51·655	39·032	12·623	0·132	+12·491	
	Cl_2	...	...	...	7·468	...	...	...	...	...	
	N	16·861	15·055	...	...	31·916	21·378	10·538	9·350	+1·188	
D_4	CaO	14·526	1·553	0·104	0·048	16·231	16·711	—0·480	0·448	—0·928	
	MgO	10·604	16·693	0·059	0·057	27·413	19·912	7·501	3·509	+3·992	
	K_2O	53·395	11·395	...	0·137	64·927	11·509	53·418	49·018	+4·400	
	Na_2O	7·579	2·991	...	6·275	16·845	7·316	9·529	3·193	+6·336	—0·15
	P_2O_5	3·140	39·693	...	...	42·833	47·066	—4·233	0·079	—4·312	
	Cl_2	11·795	0·187	...	7·442	19·424	2·108	17·316	15·179	+2·137	
	N	17·432	12·293	...	...	29·725	19·768	9·957	8·483	+1·474	
D_6	CaO	12·912	2·190	0·123	0·047	15·282	17·207	—1·925	0·191	—2·116	
	MgO	9·423	23·599	0·076	0·055	33·153	24·728	8·425	3·650	+4·775	
	K_2O	47·462	16·112	...	0·132	63·706	17·666	46·040	49·576	—3·536	
	Na_2O	6·740	4·228	...	6·073	17·041	6·978	10·063	3·480	+6·583	+1·12
	P_2O_5	2·793	56·121	...	...	58·914	46·078	12·836	0·255	+12·581	
	Cl_2	10·484	0·265	...	7·202	17·951	2·837	15·114	18·862	—3·748	
	N	15·494	17·379	...	...	32·873	23·249	9·624	12·465	—2·841	

It will be noted from Table II that lime ingestion has varied between 15 to 18 grms. while phosphate has varied from 29 to 59 grms. This has provided a ratio ranging from 1 : 2 to 1 : 4. Thus, the proportion has been too wide on one side while on the other, the phosphorus in rice *kura* is present mainly in the form of phytin which is not readily assimilated, (*vide* Discussion).

Regarding the ingestion of lime, it is no doubt higher than in Experiment A and may be considered as moderate from European standard, but due to various factors which will be discussed in due course, the lime requirement under rice straw feeding appears to be higher and the deficiency has been reflected in a negative lime-balance for eight out of nine animals. This has affected the economy of other nutrients also; for instance the amount of digestible carbohydrate assimilated by these animals has been distinctly lower than in the case of the straw and cake groups. Magee and Sen's [1931] experiment on the ratio of sugar diffusion through the walls of a rabbit's small intestine shows that when calcium is added, the diffusion increases in rate and becomes selective for glucose. Sen's [1933] later experiment with rats show that " a deficiency of calcium in the blood caused a large decrease of sugar absorption from the small intestine." Similar factors might have been responsible here.

The assimilation of other components has also been affected. Thus with about the same amount of chlorine under *aus* straw as a single feed (Experiment D) and Guinea grass (Experiment F), a positive balance has been maintained but here, eight, out of nine, have recorded a negative balance. The chlorine ingestion is, however, on the border line when compared with other combinations. Possibly the requirement under the rice-*kura*-straw combination is somewhat higher, or possibly there are other factors.

As regards phosphate although the ingestion here is high, three, out of nine, have recorded a negative balance and a close examination shows that even under a large ingestion a positive balance has only been maintained when the ingestion has been about 43 grms. phosphate. Yet this amount is far too much in excess as compared to the approximation of 10 grms. which in the case of more balanced combinations (Experiment E) seemed to be about the minimum requirement. The cause of it is most probably associated with less inorganic phosphorus and high phytin content, the effect of which (as discussed under lime and phosphate) seems to be the contributory cause.

In the case of chlorine, a striking feature has been that the urinary excretion has been very high. In fact, leaving out the urinary excretions of the Hyacinth and Napier group (Experiment F) this experiment accounts for the largest urinary excretion of chlorine both in relation to the proportion and quantity. It may be stated further that in the cases where chlorine has recorded a negative balance whether in this or other experiments urinary chlorine accounts for the major part of the excretion.

With respect to other minerals, the MgO ingestion in this experiment was the highest (except in the case of the Guinea group under experiment F) and eight, out of nine, have recorded a positive balance. The largest contribution of this mineral has been from the rice *kura*.

With respect to potash, six have recorded a positive balance and three a negative. It seems that under paddy-straw feeding, when the ingestion is below 65 or 66 grms., the tendency is for a negative balance.

In the case of soda, seven, out of nine, have recorded a positive balance.

The chief point in this experiment is, however, deficiency of lime and excess of phosphate mainly associated with rice *kura*.

Experiment C—Aman straw and one lb. linseed—cake: CaO still low though higher than in experiments A and B, MgO also low, K_2O high, and P_2O_5 protein and energy supply adequate.

The digestion tests show that the starch equivalent, nutritive ratios etc., remained nearly the same in all the four animals and during the twelve weeks over which the feeding was spread, D_1 lost a little (about 7 lb.) of its live-weight, D_4 and D_7 gained about thirty-two and seven lb., respectively, while D_3 was stationary. During the experimental period, however, all the animals exhibited some loss in live-weight probably due to the strain involved. On the whole, the feeding represented a condition of adequacy in the supply of protein and energy, so that any departure with respect to mineral metabolism was likely to be associated with the condition of the supply of the minerals in question. The main points are set up in Table III.

Table III

Aman Straw and 1 lb. Linseed-cake

Experiment C

(Computed on the basis of 500 lb. live-weight)

Animal No.	Nutrients	Intake (1933): Straw grms.	Cake grms.	Water grms.	Added mineral grms.	Total grms.	In faeces grms.	Difference grms.	In urine grms.	Balance grms.	Daily loss or gain in live-weight lb.
D_1	CaO	19·277	1·830	0·140	0·055	21·302	18·900	2·402	0·400	+2·002	—0·607
	MgO	8·327	3·620	0·080	0·065	12·092	9·400	2·692	2·260	+0·432	
	K_2O	79·275	8·000	...	0·156	87·431	21·840	65·591	64·600	+0·991	
	Na_2O	8·452	1·802	...	7·154	17·408	7·840	9·568	5·300	+4·268	
	P_2O_5	5·121	8·920	...	...	14·041	12·800	1·241	0·125	+1·116	
	Cl_2	18·361	0·183	...	8·484	27·028	10·410	16·618	14·166	+2·452	
	N	24·832	19·268	...	...	44·100	28·304	15·796	14·055	+1·741	
D_2	CaO	18·175	1·658	0·126	0·055	19·994	20·562	—0·568	0·430	—0·998	—0·133
	MgO	7·851	3·279	0·071	0·065	11·266	8·967	2·299	2·418	—0·119	
	K_2O	74·739	7·246	...	0·156	82·141	17·935	64·206	25·181	+39·025	
	Na_2O	7·968	1·632	...	7·154	16·754	5·815	10·939	35·326	—24·387	
	P_2O_5	4·828	8·080	...	...	12·908	12·138	0·770	0·100	+0·670	
	Cl_2	17·311	0·166	...	8·484	25·061	7·237	18·724	11·478	+7·246	
	N	23·552	17·453	...	...	41·005	24·432	16·573	13·523	+3·050	

D_4	CaO	19·301	1·798	0·144	0·055	21·298	21·817	—0·519	0·794	—1·313	
	MgO	8·337	3·556	0·082	0·065	12·040	9·822	3·218	3·959	—0·741	
	K_2O	79·369	7·859	...	0·156	87·384	18·468	68·916	28·585	+40·331	
	Na_2O	8·463	1·770	...	7·154	17·387	11·218	6·169	39·882	—33·713	—0·419
	P_2O_5	5·128	8·762	...	...	13·890	13·733	0·157	0·088	+0·069	
	Cl_2	18·383	0·180	...	8·484	27·047	7·505	19·542	12·448	+7·094	
	N	24·726	18·927	...	...	43·653	29·230	14·423	12·738	+1·685	
D_7	CaO	19·060	1·890	0·118	0·055	21·123	20·186	0·937	0·310	+0·627	
	MgO	8·233	3·740	0·067	0·065	12·105	9·628	2·477	3·822	—1·345	
	K_2O	73·380	8·264	...	0·156	86·800	14·814	71·986	25·723	+46·263	
	Na_2O	8·356	1·862	...	7·154	17·372	11·787	5·585	35·640	—30·055	—1·932
	P_2O_5	5·063	9·215	...	...	14·278	13·068	1·210	0·096	+1·114	
	Cl_2	18·156	0·189	...	8·484	26·829	6·612	20·217	14·774	+5·443	
	N	24·492	19·905	...	...	44·397	25·209	19·188	13·514	+5·674	

It will be seen from this table (Table III) that the amount of CaO intake ranged from 20 to 21 grms. for straw, the cake contributing hardly two grms. It seems, however, that under this combination the lime supply was either just too low or on the border line, so that there was no provision for what may be called a margin of safety. Naturally those animals which were more thrifty were able to record a positive balance, while the others could not. In this experiment, two recorded a positive balance and two a negative for CaO. The phosphate balance was uniformly positive so that the failure in the case of lime can only be ascribed to a deficiency. It, however, indicates that *aman* straw even when fed with cake requires some supplement of CaO.

The potash ingestions varied from 82 to 87 grms. and were uniformly positive, but in the case of magnesia and soda, there is some tendency of inter-relation as when magnesia is deficient, soda is negative. In this experiment, three, out of four, have recorded negative balance in MgO and Na_2O, and it seems that the deficiency of MgO is an important factor, as with about the same amount of soda intake in Experiments B and E, a positive balance has been generally recorded.

Experiment D.—Aus straw as a single feed.—CaO possibly still slightly deficient though higher than in Experiments A, B and C, low P_2O_5 and bare protein and energy.

The main data are set up in Table IV.

EXPERIMENT D

TABLE IV

Aus paddy straw as a single feed

(*Computed on the basis of 500-lb. live-weight*)

1933											
Animal No.	Nutrients	Intake				In faeces	Difference	In urine	Balance	Loss or gain in live-weight
		Straw	Water	Added mineral	Total					
		grms.	grms.	grms.	grms.	grms.	grms.	grms.	grms.	lb.
D_1	CaO	23·056	0·122	0·056	23·234	23·370	—0·136	1·051	—1·187	+0·439
	MgO	14.523	0·069	0·066	14·658	10·258	4·400	4·532	—0·132	
	K_2O	73·781	...	0·159	73·940	14·684	59·256	52·572	+6·684	
	Na_2O	7·516	...	7·300	14·816	6·908	7·908	7·340	+0·568	
	P_2O_5	6·391	...	...	6·391	6·846	—0·455	0·280	—0·735	
	Cl_2	12·200	...	8·657	20·857	10·527	10·330	6·760	+3·570	
	N	34·612	...	...	34·612	24·384	10·228	9·990	+0·238	
D_3	CaO	22·773	0·101	0·051	22·925	23·296	—0·371	0·853	—1·224	—0·442
	MgO	14·345	0·057	0·060	14·462	11·748	2·714	3·074	—0·360	
	K_2O	72·876	...	0·144	73·020	11·490	61·530	55·064	+6·466	
	Na_2O	7·424	...	6·587	14·011	5·120	8·891	4·816	+4·075	
	P_2O_5	6·312	...	...	6·312	7·283	—0·971	0·226	—1·197	
	Cl_2	12·051	...	7·812	19·863	9·406	10·457	6·965	+3·492	
	N	33·836	...	...	33·836	23·018	10·818	11·601	—0·783	

TABLE IV—(*contd.*)

Animal No.	Nutrients	Intake				In faeces	Difference	In urine	Balance	Loss or gain in live-weight
		Straw	Water	Added mineral	Total					
		grms.	grms.	grms.	grms.	grms.	grms.	grms.	grms.	lb.
	CaO	20·663	0·093	0·058	20·814	19·103	1·711	1·129	+0·582	
	MgO	13·016	0·053	0·069	13·138	6·368	6·770	4·821	+1·949	
	K_2O	66·122	...	0·165	66·287	8·133	58·154	49·325	+8·829	
D_7	Na_2O	6·736	...	7·546	14·282	2·693	11·589	3·235	+8·354	—0·422
	P_2O_5	5·727	...	...	5·727	6·750	—1·023	0·196	—1·219	
	Cl_2	10·934	...	8·949	19·883	6·004	13·879	6·648	+7·231	
	N	31·717	...	...	31·717	22·560	9·157	8·986	+0·171	

In this experiment, the intake of CaO was about 21 to 23 grms. But two animals D_1 and D_3 have given a negative balance and the same animals have also recorded a negative balance for magnesia, while P_2O_5 has been negative in all, and nitrogen in one. D_7 seems to be very thrifty as with a lower intake of CaO and MgO it has been able to maintain a positive equilibrium.

This experiment is in some respects comparable with Experiment A, in both of which the straw was fed as an exclusive feed without any concentrate. In the latter, CaO, P_2O_5, nitrogen and energy supply were all deficient, but in the former (*i.e.*, *aus* straw) P_2O_5 was definitely deficient but lime was about one and a half times as much as in Experiment A ; yet the tendency of a negative balance still persisted.

The experiment shows that up to the stage of an ingestion of 23 grms., the full requirement of CaO is still unsatisfied. It shows further that *aus* straw is superior to *aman* straw in almost all the nutrients.

Experiment E.—Aus straw and linseed cake — adequate in all nutrients and fairly representative of a complete diet for maintenance. Nutritive ratio much approximates to European standards for maintenance.

So far as energy and nutritive ratios are concerned, this experiment and Experiment C (*aman* straw and linseed cake) provide the requisite conditions. But the former differs from the latter in a better supply of mineral requirements and hence serves as an approximate comparison from varying standpoints. The CaO intake has varied from 24 to 31 grms. and the ratio CaO : P_2O_5 varied from 1 : 0·4 to 1 : 0·47, *i.e.*, CaO was about 2·1 to 2·46 times the P_2O_5. The balance figures are set up in the following table. (Table V).

TABLE V

Aus straw and Linseed cake

EXPERIMENT E

(Computed on the basis of 500-lb. live-weight)

Animal No.	Nutrients	Intake					In faeces	Difference	In urine	Balance	Loss or gain in live-weight
		Straw	Cake	Water	Added mineral	Total					
		grms.	grms.	grms.	grms.	grms.	grms.	grms.	grms.	grms.	lb.
D_2	CaO	24·554	1·251	0·148	0·055	26·003	23·903	2·100	0·947	+1·153	+0·361
	MgO	15·468	1·935	0·081	0·062	17·546	10·819	6·727	4·382	+2·345	
	K_2O	78·574	3·358	...	0·148	82·080	12·224	69·856	52·348	+17·508	
	Na_2O	8·005	1·087	...	6·800	15·892	6·206	9·686	1·882	+7·804	
	P_2O_5	6·806	3·922	...	...	10·728	10·400	0·328	0·135	+0·193	
	Cl_2	12·992	0·117	...	8·065	21·174	5·053	16·121	8·838	+7·283	
	N	36·315	9·308	...	...	45·623	25·454	20·169	12·198	+7·971	
D_8	CaO	27·846	1·465	0·167	0·061	29·539	25·697	3·842	1·180	+2·662	+0·702
	MgO	17·541	2·267	0·095	0·072	19·975	14·614	5·361	4·552	+0·809	
	K_2O	89·107	3·934	...	0·174	93·215	14·150	79·065	65·379	+13·686	
	Na_2O	9·077	1·274	...	7·967	18·318	7·449	10·869	3·782	+7·087	
	P_2O_5	7·718	4·595	...	...	12·313	11·650	0·663	0·256	+0·407	
	Cl_2	14·734	0·137	...	9·448	24·319	8·996	15·323	8·252	+7·071	
	N	41·593	10·904	...	...	52·497	31·707	20·790	12·229	+8·561	

D_3	CaO	22·404	1·091	0·146	0·046	23·687	22·071	1·616	0·710	+0·906	
	MgO	14·113	1·688	0·083	0·054	15·938	8·118	7·820	4·088	+3·732	
	K_2O	71·693	2·930	...	0·129	74·752	11·765	62·987	42·305	+20·682	
	Na_2O	7·304	0·949	...	5·932	14·185	4·625	9·560	0·920	+8·640	+0·580
	P_2O_5	6·210	3·421	...	...	9·631	10·613	—0·982	0·211	—1·193	
	Cl_2	11·855	0·102	...	7·035	18·992	9·270	9·722	4·179	+5·543	
	N	33·020	8·119	...	...	41·139	23·222	17·917	15·267	+2·650	
D_4	CaO	26·380	1·812	0·152	0·051	28·395	27·334	1·061	0·915	+0·146	
	MgO	16·617	2·804	0·086	0·060	19·567	14·848	4·719	4·477	+0·242	
	K_2O	84·414	4·867	...	0·144	89·425	13·783	75·642	64·242	+11·400	
	Na_2O	8·599	1·576	...	6·612	16·787	6·649	10·138	2·292	+7·846	+0·499
	P_2O_5	7·311	5·684	...	...	12·995	11·258	1·737	0·226	+1·511	
	Cl_2	13·958	0·114	...	7·841	21·913	12·370	9·543	5·676	+3·867	
	N	38·594	13·489	...	...	52·083	30·508	21·575	15·785	+5·790	

TABLE V—(*contd.*)

Animal No.	Nutrients	Intake: Straw grms.	Intake: Cake grms.	Intake: Water grms.	Intake: Added mineral grms.	Intake: Total grms.	In faeces grms.	Difference grms.	In urine grms.	Balance grms.	Loss or gain in live-wieght lb.
	CaO	28·755	2·047	0·157	0·057	31·016	28·488	2·528	1·075	+1·453	
	MgO	18·135	3·167	0·089	0·068	21·459	11·629	9·830	5·136	+4·694	
	K_2O	82·789	5·497	...	0·163	88·449	12·149	76·300	73·382	+2·918	
D_2 .	Na_2O	9·327	1·780	...	7·468	18·575	5·177	13·398	3·058	+10·340	+0·282
	K_2O_5	7·890	6·420	...	...	14·310	13·034	1·276	0·148	+1·128	
	Cl_2	13·157	0·128	...	8·856	22·141	13·502	8·639	2·959	+5·680	
	N	41·790	15·235	...	...	57·025	30·725	26·300	18·658	+7·642	
	CaO	24·098	1·773	0·174	0·050	26·095	23·848	2·023	0·745	+1·278	
	MgO	16·006	2·743	0·099	0·059	18·907	11·972	6·777	3·427	+3·350	
	K_2O	72·947	4·761	...	0·141	77·849	16·670	61·038	58·273	+2·765	
D_3 .	Na_2O	7·431	1·542	...	6·468	15·441	3·530	12·155	0·896	+11·259	
	P_2O_5	6·715	5·561	...	...	12·276	11·232	1·044	0·164	+0·880	+0·434
	Cl_2	11·473	0·111	...	7·671	19·255	11·614	7·652	2·990	+4·662	
	N	36·337	13·196	...	...	49·533	27·283	22·250	16·498	+5·752	

It will be seen from Table V that CaO, MgO, K_2O, Na_2O and Cl_2 have uniformly maintained a positive figure in all the six animals under experiment. In the case of P_2O_5 also a positive balance has been kept in five out of six. Only one has recorded a negative balance, and this provides a very interesting study. This animal (D_9) was the heaviest amongst the lot and weighed 603 lb. during the experimental period. It had received about half lb. of cake which when worked out on the basis of 500-lb. live-weight only provided 3·421 grms. P_2O_5 from cake as compared to higher amounts in the case of other animals. The result was that while the other animals received P_2O_5 to the amount of 11 to 14 grms. from the total feeds (cake and straw), the amount falling to the share of this animal was 9·63 grms. only. This is highly suggestive that the minimum P_2O_5 requirements under these conditions of feeding is about 10 grms. per 500-lb. live-weight. It would be worth while to quote here the remark of Theiler and Green [1932] that " when the phosphorus intake of an adolescent bovine drops to the vicinity of twenty grms. P_2O_5 per day on a natural pasture, fatal aphosphorosis will develop in due course." This suggests a requirement of 10 grms. P_2O_5 per 500-lb. live-weight and is corroborative of our findings.

Experiment F—Aman straw and green feeds. As already stated this experiment is really divided into three parts—Hyacinth group, Napier group and Guinea-grass group.

At the outset it is necessary to state that it would have been better if some more work with these feeds could have been done before incorporating this part here. On the other hand, some of the results appear to be highly suggestive and their inclusion has been prompted from this consideration. These are submitted subject to this reservation.

The main features in this experiment (Table VI) are an ingestion of high CaO, high K_2O with low P_2O_5 (and also nitrogen) in the Hyacinth group ; low CaO and high K_2O in the Napier group ; and high CaO, medium K_2O and possibly high Na_2O in the Guinea-grass group.

In the case of the Hyacinth group, on account of a very low ingestion of P_2O_5 (6·5 to 7 grms.) the ratio CaO : P_2O_5 has varied from 7·34 to 9·02, *i.e.*, lime has been seven to nine times as much. There is also further imbalance with respect to high potash and low nitrogen. The chlorine ingestion has been similar to that of Experiment B (*aman* straw and *kura*) and like it has recorded a negative balance. The potash ingestion is, however, very high—in fact the highest with the exception of the Napier group, yet a definitely negative balance in the two animals experimented with has been recorded. As for CaO, in spite of heavy ingestion (48 to 64 grms.), the animals have not been able to effect a definitely positive balance. Animal D_2 has recorded a negative balance in CaO, K_2O, Na_2O, P_2O_5, Cl_2 and nitrogen. Since the low ingestion will necessarily be associated with negative balance, the results under P_2O_5, Cl_2 and N might be what was expected. But the cases of CaO and K_2O are different as both of them were ingested in heavy doses, yet K_2O

was all negative while half of CaO was positive and half negative. This might be due to the resultant effect of a general imbalance under which this feeding was conducted. The results under Guinea-grass and Napier groups, however, suggest that, in the Hyacinth group, although the general imbalance might be the aggravating cause, the very high ingestion of lime by itself or potash by itself may also develop a condition of disturbed metabolism. Thus, in the case of Guinea-grass, there was otherwise a liberal supply of almost all components whether with respect to protein and energy or minerals. But CaO ingestion was considerably in excess as compared to all other experiments (except Hyacinth group), yet the results have divided into half positive balance and half negative. In other words, in spite of such heavy ingestion there was as much chance of positive balance as negative.

It should be stated that from Experiment E, it would appear that a minimum dose of 24 grms. CaO is likely to effect a positive balance. On this basis, the amount in the case of the Hyacinth and Guinea-grass groups, has varied from two to two and a half times as much. Such a large amount is not only uneconomic by itself (as it would mean a waste) but is likely to react adversely on the metabolism of other nutrients. There is thus an illustration of this (as indicated from the negative balance) in the case of both the Hyacinth and the Guinea-grass groups, and even if the former is excluded on account of a general imbalance in other respects, the behaviour of the Guinea-grass bears out that even when other nutrients are well supplied, a faulty proportion in lime, potash and possibly soda, is undesirable. Thus the heavy ingestion of CaO has not only exhibited a tendency of negative balance by itself but has also reacted on the K_2O metabolism. It may be stated that with a similar dose of potash in Experiments B and D, a positive balance has generally been obtained.

Turning now to the Napier group, it is very low in lime and very high in potash. In fact, the potash ingestion has been the heaviest of all other feeds being as much as 156 to 173 grms. per 500-lb. live-weight. But in spite of this one has recorded a negative balance and this combined with low lime ingestion has also reacted on the phosphate metabolism resulting in negative balance in the case of D_5 which has recorded negative balance for CaO, MgO, K_2O, P_2O_5 and N.

The results obtained from the rather limited data on green feeds may be tentatively summed up as follows :—

Guinea-grass is better balanced than Napier and Hyacinth, but it is not advisable to feed it *ad lib* as this entails a heavy ingestion of CaO, nitrogen and soda. This is wasteful and might probably also be detrimental. Napier and Hyacinth should on no account be fed *ad lib.*, except when the former has been supplemented with CaO and both have had an addition of cake. Hyacinth should always be given in regulated doses, the deficiencies being made up. Napier is quite rich in P_2O_5 but Hyacinth is very poor. Hyacinth is really not a cattle feed but has sometimes to be used in cases of fodder scarcity.

EXPERIMENT F.

TABLE VI

Aman straw and green feed

(Computed on the basis of 500-lb. live-weight)

1932

Feed	Animal No.	Nutrients	Intake: Straw grms.	Intake: Green feed grms.	Intake: Added mineral grms.	Intake: Total grms.	In faeces grms.	Difference grms.	In urine grms.	Balance grms.	Loss or gain in live-weight lb.
Aman straw and water-hyacinth	D₁	CaO	6·296	58·229	...	64·525	52·770	11·755	1·357	+10·398	—2·281
		MgO	4·553	13·176	...	17·729	13·117	4·612	4·429	+0·183	
		K_2O	24·726	81·406	...	106·132	14·780	91·352	93·888	—2·536	
		Na_2O	5·557	2·560	10·606	18·723	8·080	10·643	8·220	+2·423	
		P_2O_5	2·181	4·970	...	7·151	9·593	—2·442	0·079	—2·521	
		Cl_2	3·046	4·791	11·689	19·526	11·779	7·747	54·200	—46·453	
		N	7·269	14·750	...	22·019	22·529	—0·510	10·069	—10·579	
	D₂	CaO	6·100	41·609	...	47·709	48·137	—0·428	2·411	—2·839	+0·971
		MgO	4·411	11·633	...	16·044	11·525	4·519	4·130	+0·389	
		K_2O	23·957	71·876	...	95·833	8·830	87·003	90·452	—3·449	
		Na_2O	5·384	2·261	9·872	17·517	7·641	9·876	12·783	—2·907	
		P_2O_5	2·114	4·388	...	6·502	8·802	—2·300	0·084	—2·384	
		Cl_2	2·951	4·230	10·880	18·061	...	...	65·425	...	
		N	7·044	12·727	...	19·771	19·783	—0·012	9·069	—9·082	

TABLE VI—(*contd.*)

Feed	Animal No.	Nutrients	Intake: Straw	Intake: Green feed	Intake: Added mineral	Intake: Total	In faeces	Difference	In urine	Balance	Loss or gain in live-weight
			grms.	g ms.	grms.	grms.	grms.	grms.	grms.	grms.	lb.
Aman straw and Napier grass	D4	CaO	4·829	14·778	...	19·607	17·712	1·895	0·977	+0·918	—0·305
		MgO	3·492	11·956	...	15·448	9·926	5·522	4·276	+1·246	
		K_2O	18·969	153·899	...	172·868	23·107	149·761	133·453	+16·309	
		Na_2O	4·263	9·520	8·286	22·069	6·982	15·087	10·246	+4·841	
		P_2O_5	1·674	25·772	...	27·446	26·712	0·734	0·140	+0·594	
		Cl_2	2·336	18·304	9·132	29·772	2·100	27·672	36·607	—8·935	
		N	5·449	27·666	...	33·115	20·183	12·932	11·629	+1·303	
	D5	CaO	4·648	13·084	...	17·732	16·706	1·026	1·049	—0·023	—0·857
		MgO	3·361	10·587	...	13·948	10·718	3·230	4·481	—1·251	
		K_2O	18·255	136·265	...	154·520	27·758	126·762	159·256	—32·494	
		Na_2O	4·103	8·430	8·304	20·837	5·107	15·730	7·896	+7·834	
		P_2O_5	1·611	22·820	...	24·431	27·884	—3·453	0·139	—3·592	
		Cl_2	2·248	16·207	9·152	27·607	3·433	24·174	30·738	—6·564	
		N	5·367	24·497	...	29·864	21·352	8·512	13·669	—5·157	

Aman straw and Guinea-grass	D_3	CaO	2·982	56·917	...	59·899	57·100	2·799	0·267	+2·532	
		MgO	2·157	31·155	...	33·312	27·555	7·757	6·510	+1·247	
		K_2O	11·713	62·095	...	73·808	11·420	62·388	69·391	—7·003	
		Na_2O	2·632	24·479	7·915	35·026	14·863	20·163	17·349	+2·814	—1·108
		P_2O_5	1·034	23·151	...	24·185	20·827	3·358	0·117	+3·241	
		Cl_2	1·442	7·917	8·723	18·082	1·406	16·676	15·597	+1·079	
		N	3·444	54·579	...	58·023	25·580	32·443	25·075	+7·368	
	D_6	CaO	2·514	52·421	...	54·935	55·144	—0·209	1·149	—1·358	
		MgO	1·817	30·976	...	32·793	23·043	9·750	7·973	+1·777	
		K_2O	9·872	61·739	...	71·611	9·699	61·912	82·493	—20·581	
		Na_2O	2·219	24·339	7·180	33·738	20·236	13·502	15·192	—1·690	+0·376
		P_2O_5	0·871	23·019	...	23·890	21·614	2·276	0·173	+2·103	
		Cl_2	1·216	7·871	7·913	17·000	1·182	15·818	13·714	+2·104	
		N	2·902	54·266	...	57·168	28·064	29·104	27·292	+1·812	

Discussion

In the preceding pages, thirty individual data have been arranged and described under six heads of Experiments (A to F). They represent investigations commencing from the stage of deficiency (as for instance Experiments A, B and C for CaO) and rising to the stage of adequacy (Experiment E). Some have exhibited instances of heavy ingestion, as for instance P_2O_5 in Experiment B and CaO in parts of Experiment F. The combinations were drawn up mainly in conformity with the nature of the feeds often used in these parts of India.

A study of these data will show that in all combinations except the green feed groups (Experiment F), rice straw has been the chief source of CaO and K_2O while P_2O_5 has been mainly derived from the concentrates—cake and *kura*. Rice straw has also been the main source of magnesia except in the combination with rice *kura* (Experiment B) in which the major part was derived from the latter—about half to three-fourths of the total. In the case of combinations with green feeds the greater proportions of CaO, MgO, and K_2O on one side and P_2O_5 on the other, were derived from the green fodders. Guinea-grass and Hyacinth have been responsible for the heaviest ingestion of CaO, while Napier accounts for the heaviest ingestion of K_2O seconded by Hyacinth. Guinea-grass also accounts for the highest ingestion of soda. It also provided a liberal protein supply which exceeded some of the cake combinations under Experiments C and E. Napier grass appears to be poor in CaO (0·4 per cent on dry basis) and although the fodder was consumed in large amounts, the CaO derived from it was naturally low.

If the different tables are gone through (I to VI) set up in the different experiments, it may be noted that the results on balances provide an approximate indication of the mineral requirement. Since lime and phosphate are the main components, the aspect of their requirements may first be considered.

Lime requirements

As already stated the results provide data from the stage of deficiency to that of sufficiency; and this is more strikingly noticeable with respect to the behaviour of CaO. The computations on the basis of 500-lb. live-weight also provide a more rational comparison. If the intakes of all experiments are assembled with the symbols of their positive and negative retentions (Table VII) one can see at a glance that starting from Experiment A up to Experiment D, the intakes of CaO have gone up from 14 grms. to 23 grms.

TABLE VII

Intake of Mineral Nutrients

(Computed on the basis of 500-lb. live-weight)

Particulars of Experiments	Animal No.	Live-weight during the experimental period lbs.	CaO grms.	MgO grms.	K_2O grms.	Na_2O grms.	P_2O_5 grms.	Cl_2 grms.	N grms.
Aman straw only Experiment A	D_7	343	14·707—	10·636+	57·765—	23·806+	5·098—	19·042—	16·981—
	D_8	335	13·473—	9·743+	52·919—	22·975+	4·670—	18·730+	15·557—
Aman straw and rice *kura* Experiment B	D_1	550	18·024+	29·516+	74·950+	16·999+	44·446+	18·909—	33·851+
	D_5	536	18·227—	27·387+	74·688+	17·210+	38·843—	19·364—	32·139+
	D_7	533	15·394—	30·104+	66·384+	15·839—	50·169+	17·296—	33·604+
	D_8	512	17·458—	28·924—	71·868+	17·102+	38·906—	19·218—	31·856—
	D_9	612	16·795—	29·112+	70·599+	14·058+	45·465+	10·609—	32·946+
	D_2	585	14·207—	32·227+	59·876—	16·525+	58·282+	17·179—	31·534+
	D_3	568	16·090—	30·805+	65·771—	17·293—	51·655+	...	31·916+
	D_4	570	16·231—	27·413+	64·927+	16·845+	42·833—	19·424+	29·725+
	D_6	589	15·282—	33·153+	63·706—	17·041+	58·914+	17·951—	32·873—

TABLE VII—(*contd.*)

Particulars of Experiments		Animal No.	Live-weight during the experimental period lbs.	CaO grms.	MgO grms.	K_2O grms.	Na_2O grms.	P_2O_5 grms.	Cl_2 grms.	N grms.
Aman straw and 1-lb. cake Experiment C		D_1	500	21·302+	12·092+	87·431+	17·408+	14·041+	27·028+	44·100+
		D_3	552	19·994—	11·266—	82·141+	16·754—	12·908+	25·961+	41·000+
		D_4	509	21·298—	12·040—	87·384+	17·387—	13·890+	27·047+	43·653+
		D_7	484	21·123+	12·105—	86·800+	17·372—	14·278+	26·829+	44·397+
Aus straw only Experiment D		D_1	490	23·234—	14·658—	73·940+	14·816+	6·301—	20·857+	34·612+
		D_3	543	22·925—	14·462—	73·020+	14·011+	6·312—	19·863+	33·836—
		D_7	474	20·814+	13·138+	66·287+	14·282+	5·727—	19·883+	31·717+
Aus straw and cake Experiment E		D_2	526	26·003+	17·546+	82·080+	15·892+	10·728+	21·174+	45·623+
		D_8	449	29·538+	19·975+	93·215+	18·318+	12·313+	24·319+	52·497+
		D_9	603	23·687+	15·938+	74·752+	14·185+	9·631—	18·992+	41·139+
		D_4	541	28·395+	19·567+	89·425+	16·787+	12·995+	21·913+	52·083+
		D_5	479	31·016+	21·459+	88·449+	18·575+	14·319+	22·141+	57·025+
		D_6	553	26·095+	18·907+	77·849+	15·441+	12·376+	19·255+	49·533+
Aman straw and green feed Experiment F	Hyacinth group	D_1	350	64·525+	17·729+	106·132—	18·723+	7·151—	19·526—	22·019—
		D_2	376	47·709—	15·044+	95·833—	17·517—	6·502—	18·061—	19·771—
	Napier group .	D_4	448	19·607+	15·448+	172·868+	22·069+	27·446+	29·772—	33·115+
		D_5	447	17·732—	13·948—	154·520—	20·837+	24·431—	27·607+	29·864—
	Guinea group .	D_3	469	59·899+	33·312+	73·808—	35·026+	24·185+	18·082+	58·023+
		D_6	517	54·935—	32·793+	71·611—	33·738—	23·890+	17·000+	57·168+

In Experiment A (with 14 grms. intake) all are negative, in Experiment B (15 to 18 grms. intake) eight, out of nine, are negative, in Experiment C (19 to 21 grms. intake) two, out of four, are negative, in Experiment D (21 to 23 grms. intake) two, out of three, are negative. In other words up to the stage of 23 grms. ingestion of CaO, the general tendency has been definitely towards negative balance. It is only when the ingestion has reached about 24 grms. and upwards that a definite positive balance has been maintained. It would, therefore, be a reasonable assumption that *under the condition of rice straw feeding the minimum lime requirement is about 24 grms. per 500-lb. live-weight.*

According to Kellner [1915] "the daily consumption of 50 grms. phosphoric acid and 100 grms. lime per 1,000-kg. live-weight sufficiently meet all requirements". This works out at 22·7 grms. CaO for 500-lb. live-weight.

In the experiments carried out elsewhere, it will be noted that in Diakow's experiment * [1913] the minimum of 0·115-lb. calcium (equivalent to 79·04 grms. CaO) sufficed "to support a not inconsiderable gain". On the basis of 500-lb. live-weight this is equivalent to 34·54 grms. CaO.

In Cochrane's experiment,† 0·147-lb. calcium (equivalent to 93·44 grms. CaO) resulted in gain. In Henneberg's investigations ‡ [1860] upon the maintenance of cattle a distinctly smaller amount, *viz.*, 0·09-lb. Ca (equivalent to 57·20 grms. CaO per 1,000-lb. live-weight) proved adequate for maintenance. This is equivalent to 28·6 grms. CaO per 500-lb. live-weight. Wellmann [1931] gives the maintenance requirement at 50 to 100 grms. CaO per 1,000-kg. live-weight, which is equivalent to 11·35 to 22·7 grms. CaO per 500-lb. live-weight.

In India, experiments in these lines were mainly conducted by Warth and his associates. In one of their experiments [1932] with *Bolarum* hay and *ragi* straw the ingestion of CaO (in the case of no supplement) varied from 34 to 46 grms. for the former and 52 to 56 grms. for the latter. The supply appeared to be fairly adequate from European standard, but the intake of phosphoric acid was definitely low and the authors remarked that "lime assimilation fluctuated according to the amount present in the food" and that some loss of lime "was associated with and in some way brought about by the loss of phosphoric acid". In subsequent trials with *jowar* hay and rice straw conducted by two of his associates [Iyer and Krishna Ayyar, 1934], the lime intake varied from 14 to 18 grms. in *jowar* hay and 22 to 27 grms. in rice straw, yet while the *jowar* hay recorded positive balance, two, out of three, under rice straw gave negative results. The authors have tabulated the two years' averages from which the following is taken.

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* Diakow. Landw. Jahrb. **44,** 833.
† Cochrane. Penna. Inst. An. Nutrn. unpublished results.
‡ Henneberg. Beitrag, etc., Heft, 1 (1860) p. 113.
} cited by Armsby.

Food	CaO		P_2O_5	
	Intake grms.	Balance grms.	Intake grms.	Balance grms.
Bolarum hay	41·17	+0·08	6·64	—3·18
Ragi straw	57·99	+2·30	12·72	+0·05
Jowar hay	16·02	+2·62	17·70	+3·11
Rice straw	24·91	—2·42	11·14	+0·09

The authors' inference was that "if the ration provides a sufficient excess of lime there will be some assimilation even if the phosphoric acid content is low" and further that the "*jowar* figures show that even when lime is low, assimilation can occur if excess of phosphoric acid is present". The main question that arises from it is: Can assimilation be possible if the supply is below the minimum? To the present authors it appears that minimum supply must necessarily be the first condition, and thereafter the assimilation is more intimately associated with the kind and quality of the fodder. This point will be apparent from a study of the behaviour of different fodders; and if the case of rice straw (with which this paper is more directly concerned) is taken, it is noted that the assimilation of lime from it was much poorer; and that there is a certain definite stage below which the liability of negative assimilation is very great.

The point of interest is that the minimum amount corresponding to this stage is much higher than what is generally assumed to be adequate with many feeds. The figures recommended by Kellner [1915] and Wellmann [1931] have been already cited. Huffman [1934] prescribes 10 grms. Ca or 14 grms. CaO. One of the Bangalore workers [Iyer, 1935] found on the basis of another series of trials with Rhodes grass hay, Aurangabad hay, spear grass hay and *jowar* hay that "the actual minimum values found.............are 10 grms. phosphoric acid and 15 grms. calcium oxide for an animal of 750-lb. live-weight". These values are much low as compared to those found under rice straw feeding. The tests at Dacca were conducted on a comprehensive scale and the results definitely indicate that rice straw provides certain conditions under which lime assimilation does not proceed as satisfactorily as in many other feeds. The tests at Bangalore, although confined to three only, also point to similar indications as will be noted from the remark (*loc cit*) that "for some reasons lime is not very readily assimilated from rice straw".

In the absence of direct evidence it is difficult to assign any definite reason for this, but if the figures under rice straw feeding are carefully studied it is noted that rice straw involves a very large ingestion (about 70 to 80 grms. per 500-lb. live-weight) of potash. This is generally much in excess as compared to what is ingested through many of the other feeds. Orr [1925] considers that " the ratio of sodium to potassium affects the assimilation of both calcium and phosphorus ". Godden [1928] says that his work in conjunction with Richards and Husband has shown that an excess of potassium in the ration while temporarily depleting the sodium has its main effect " on the nitrogen, calcium and phosphorus balances; a high ratio of $K_2O : Na_2O$ tends to lower the assimilation of these constituents, whereas when the sodium is in excess the assimilation is raised " and he remarks that this " may be the explanation of the beneficient results of feeding sodium chloride to stock ". Remy and Müller [1931] have found in rats that a diet with a high content of potassium results in rachitic symptoms and they ascribe it not to high potassium intake but to the consequent diminution of the Ca : K ratio. Armsby states [1917] " that fodders that cause malnutrition of the bones resulting in the disease known as rickets (Rachitis) usually show a misproportion of potassium to sodium " and he cites figures from Zuntz * [1917] of such fodders (hay) causing the disease.

It need hardly be stated that very little work has been done on the aspects of calcification under cereal straw feeding. Much work has, however, been done with cereal diets and various investigators have noted a deleterious effect upon the calcification of bones and teeth in dogs and rats when fed with cereal diets. E. Mellanby [1926] proposed the idea that cereals contain some distinct anti-calcifying substance which he provisionally called a toxamine. Mellanby [1928, 1929] noticed that those cereals which frequently contained the most Ca and P had the worst effect on teeth and that Ca/P ratio was not found responsible. Holst [1927] reported a ricket-producing factor from oats which could be extracted with 0·5 per cent HCl and Mirvish [1929, 1930] reported that when a dilute HCl extract of oats was injected into animals it produced a marked fall in blood calcium. Fine [1930] ascribed the difference in the calcifying properties to variations in the vitamin D content. Lowe and Steenbuck [1936] found that the inorganic phosphorus content of variously treated samples of maize bore a direct relation to the anti-rachitic effectiveness of the ration and an inverse relation to the phytin content, and the hydrolysis of phytin by HCl improved it to the extent of hydrolysis.

It is very difficult to say how far similar probabilities are associated with the feeding of cereal straw. But there can be no doubt that the comparatively larger requirement of lime under rice straw feeding must be due to a poorer economy. It has been suggested by Hunter and his co-workers [cited by Godden,

* Zuntz. 1912 Jahrb. Dent. Landw. Gessell, 577, quoted by Armsby (21), 342.

1928] that the calcium in green food and carefully-won hay is in a more highly dispersed condition, hence better digested and assimilated than in dry hay. Their suggestion is based on the positive results obtained by feeding tricalcium phosphate precipitated on starch. Possibly the lime compound in the rice straw is in a coarser aggregation and the presence of a larger quantity of potassium salt accompanied with other factors complicates the matter further.

There might be another factor, *viz.*, deficiency of vitamin as suggested by Fine [1930], but Godden [1928] remarks that feeding experiments conducted at the Rowett Institute indicate that, on a properly balanced ration, with the animals kept under normal conditions in respect of sun light and exercise, there is but little risk of disease due to deficiency of vitamins. Other investigators such as Warth [1932] and Theiler, Green and du Toit [1927] also think that in the case of herbivora the evidences are inconclusive on this point.

The chief facts that emerge out from rice straw feeding are—

(1) that lime assimilation is poor,

(2) that positive balance cannot be attained until the intake is about 24 grms. CaO per 500-lb. live-weight.

(3) that this requirement is higher than the generally prescribed standard,

(4) that rice straw involves a comparatively larger ingestion of potash on account of which, according to various investigators, lime assimilation might be adversely affected.

It need only to be stated here that if 24 grms. CaO per 500-lb. live-weight represent the minimum requirement under rice straw feeding, a somewhat higher amount would be essential in actual practice. Kellner [1915] says that only one-third to one-half of P_2O_5 and CaO can be taken from vegetable foods so that two to three times as much must be given as can be stored in the body. Possibly the amount if given in pure form, say as calcium carbonate or phosphate, would be different. According to Hawk [1931], a 50 per cent higher calcium level is assumed as providing a " margin of safety " in the case of American dietaries (human). On such basis the requirement under rice straw feeding would be about 36 grms. per 500-lb. The point can only be established after further trial and it is proposed to take it up according as facilities are available.

Before winding up the discussion on lime requirement, it is necessary to state that an excess intake over and above the requirement is not only wasteful but may possibly be injurious. The behaviour of animals under the green feeds (Experiment F) with regard to Hyacinth and Guinea grass groups are indications of this. In these the ingestion of lime has been very large, *viz.*, 48 to 64 grms. but in spite of it half the results have recorded negative balance. In the case of Hyacinth there might be some justification as the feed was rather

unusual, but the same cannot be stated of Guinea grass. It is more likely that excess of lime is probably not well tolerated, as it would appear from the investigations of Cox and Imboden [1936] who found in their experiment on reproduction in rats that excess of phosphorus was better tolerated than excess of lime.

The investigation of Meigs and his co-workers [1926] on lactating cows show that an excess of calcium might interfere with phosphorus assimilation and that two parts or more by weight of calcium to one of phosphorus constituted an excess. They also point out from their work that a long continued loss of calcium will cause a loss of phosphorus even though the ration may contain plenty of assimilable phosphorus. The case of D_5 under Napier group in Experiment F typically represents this condition (*vide* also discussion under phosphate). They further obtained results indicating that calcium is very poorly assimilated from hay which was exposed to rain. This latter point is of much importance to Bengal and more or less to whole of India where the straw is almost invariably stacked in the open. Particular care should be bestowed to guard against defective stacking by which the quality of straw will deteriorate, thereby reacting on the lime and other mineral content and also on assimilation.

Phosphate requirement

This aspect has been partially dealt with under Experiment E. If the various combinations as set forth in Table VII are studied, it will be noted that the ingestion of P_2O_5 from them has varied from 4·67 grms. (Experiment A) to 58·91 grms. (Experiment B). The results may be divided under the following :—

(1) those coming under definite deficiency (Experiments A, D, and Hyacinth group under Experiment F),

(2) those standing on the border line (animals D_2 and D_9 under Experiment E),

(3) those providing adequacy (Experiment C and animals D_8, D_4, D_5 and D_6 under Experiment E),

(4) those exceeding the stage of adequacy (Napier and Guinea grass groups under Experiment F), and

(5) those deviating from normal behaviour (Experiment B).

The results under the deficiency groups of Experiments A and D represent a condition under which the animals in Bengal are sometimes or often maintained. They show in particular that under the Bengal condition of feeding there is a greater liability of phosphorus deficiency than that of lime. A small supplement of cake or material rich in assimilable phosphorus can appreciably improve the condition, and attention should be mainly concentrated towards impressing this point.

Turning now to the next groups, if the intakes (Table VII) are followed from the stage where the figures stand on the border line it will be noted that the first minimum intake coincident with positive balance has been in the case of animal D_2 which had ingested 10·728 grms. P_2O_5 (Experiment E); and the noteworthy point is that all ingestions above this quantity (excluding of course the groups departing from normal behaviour) have been attended with a definite positive balance.

This particular experiment (Experiment E) represents a condition of adequacy in virtually all nutrients as will be borne out from the results of balances. There were six animals under it and five of them recorded positive balances in all nutrients. The sixth one (animal D_9) also behaved identically in all other respects, except in P_2O_5 in which only it recorded a negative balance. This animal along with D_2 and D_8 belonged to the ½-lb. linseed-cake group and received like them about 11·2 grms. P_2O_5; but while the other two definitely recorded positive balance this one failed to do so. On closer examination, it was noted that this animal was the heaviest of the whole lot and weighed 603-lb. When, therefore, the ingestion was computed on the basis of 500-lb. live-weight, it worked out at 9·631 grms. P_2O_5 or less than 10 grms. It will be seen that there is not a single instance in which an ingestion below this amount has been followed with a positive balance whereas all ingestions above this amount have been definitely positive. This amount thus constitutes the limiting factor for proper assimilation and we are provided with a definite suggestion that the minimum P_2O_5 requirement is about 10 grms. per 500-lb. live-weight or 20 grms. per 1,000-lb.

It will be interesting to refer to the findings of other workers. The opinion of Theiler and Green has been already cited [Theiler and Green, 1932], *viz.*, that "fatal aphosphorosis will develop" when the phosphorus intake drops to the vicinity of 20 grms. In the earlier work of Theiler and his co-workers [1927] they found that with cattle grazing on the veld a daily consumption of about 11·79 grms. of phosphorus was regarded as somewhere about the point at which osteophagia could develop or disappear in grazing cattle of 1,000-lb. live-weight. Kellner prescribes 50 grms. per 1,000-kg. live-weight. This works out at 11·35 grms. per 500-lb. Armsby [1917] computed from Lawes and Gilbert's analysis of the ash of the entire body of farm animals, that during the first year cattle retain 8·14 grms. phosphorus per day. This figure agrees with Kellner's [1914] who computed the phosphorus retention of growing calves as 8·3 grms. per day during the first year. Wellmann [1931] has given the maintenance requirement at 25 to 50 grms. per 1,000-kg. or 5·675 grms. to 11·35 grms. per 500-lb. live-weight. Huffman and his co-workers [1933] found that 5·7 to 9·9 grms. phosphorus per day was insufficient (under sun-cured alfalfa hay and phosphorus ration 4 : 1 to 5 : 1) but 10 to 12 grms. furnished sufficient phosphorus for

maintenance, normal growth, and for the development of the foetus from eighteen months of age to first calving. They suggest that " at least 17 grms. of phosphorus be allowed daily for low milk production and the dry period during the advanced stage of gestation ". Rose [1912] concluded that the necessary amount to maintain phosphorus equilibrium in milking cows appeared to be the amount eliminated in milk *plus* 26 mg. per kgm. of body-weight. Evidently the latter (26 mgm.) represented the maintenance requirement and is equivalent to 26 grms. per 1,000-kg. live-weight.

Turning now to the results of Dacca experiments it may be said that when 10 grms. P_2O_5 per 500-lb. live-weight constitutes the limiting factor under rice straw feeding, it will be advisable to follow the American practice (as suggested under lime) and add 50 per cent to it in order to provide a margin of safety. This works out the minimum requirement at 15 grms. phosphate per 500-lb. live-weight or 30 grms. per 1,000-lb.

If the amount is to be fed as a mineral supplement it is well to bear in mind the interesting theory advanced by Meigs *et al* [1926] *viz.*, that assimilation is facilitated by feeding calcium and phosphorus alternately. According to them the absorption of phosphorus from the intestinal tract is hindered by the simultaneous presence of calcium compound. They suggest from their metabolic tests that the phosphorus assimilation by pregnant cows and " probably that of calcium also " is favoured by adding di-sodium phosphate to the ration and feeding it on alternate days.

Phosphorus ingestion exceeding the stage of adequacy

The experiments bearing on this were in one case limited to four animals (Napier and Guinea grass groups under Experiment F) and in another (Experiment B under rice *kura* group) it was governed by other factors for which it does not strictly fall into this category, and will be dealt with separately. In the former case the ingestion of P_2O_5 was from 24 grms. to 27 grms. and was not at any rate so large as could be considered unusual or excessive. Such ingestion may be uneconomical but may not necessarily be harmful. On these data little can be said of the possible effect of a truly excessive ingestion.

There is, however, an interesting side issue which deserves attention. One of the animals D_5 under Napier feeding recorded a negative balance in spite of such a large ingestion of P_2O_5. Its explanation probably lies in the fact that Napier grass involved a very heavy (in fact the heaviest) ingestion of potash (155 to 173 grms. K_2O) while the lime ingestion was only 18 to 20 grms. CaO. The behaviour of a large ingestion of potash and its adverse effect on lime assimilation has been partially discussed and will also be further discussed under potash. It has been pointed out that under rice straw feeding (with which a large quantity

of potash is ingested) the lime requirement has been found to be higher. In the case of Napier the condition is probably further aggravated as the potash ingestion is considerably higher while the low lime ingestion does not provide any margin to ensure a definite positive balance. This is typically reflected in the behaviour of animal D_5 which probably suffered systematically from a faulty lime assimilation which adversely reacted on phosphate balance in spite of a high P_2O_5 intake. It has been pointed out, as already stated under lime by Meigs *et al* [1926], that a long continued loss of lime will cause a loss of phosphorus even though the ration may contain plenty of assimilable phosphorus as is normally expected in a green feed (*vide* also under lime). It is very striking in this animal (Table VII) that lime, magnesia, potash, phosphorus and nitrogen were all negative. Only soda and chlorine were positive.

Phosphorus ingestion in experiments exhibiting deviation

It remains now to take up the results of rice *kura* in which as already stated, the conditions are probably governed by other factors. The phosphorus content in the rice *kura* is very large (about 6 per cent in the sample used) and naturally the ingestion in individual animals were far more than in any other feed. This experiment would thus have served as a suitable example of excessive feeding of P_2O_5 but due to other factors the conditions have been different. The phosphorus in rice *kura* is largely in the form of phytin. Winton and Winton [1932] cite from Suzuki, Yoshimura and Takaishi that rice bran contains about 8 per cent phytin. The same authors also report on the work of Rather [1918] that of the total soluble phosphorus about 87 per cent and 93 per cent were found in rice bran and rice polish, respectively, as inosite pentaphosphoric acid. It has been already stated that the feeding of rice bran was followed by low appetite, loss of condition and so forth. This is in all probability associated largely with the nature of phosphorus compound in the feed as would appear from the work of various investigators. Thus Bruce and Callow [1934] reported that " the apparent rachitogenic effect of cereals when compared with other materials of the same phosphorus content is due to the fact that cereal phosphorus is not in an available form ". They expressed that " the differences between oat-meal, maize and white-flour can be completely accounted for by differences in the total phosphorus content and in the proportion of inositol—hexa-phosphoric acid ". McChance and Widdowson [1935] fed phytin to three adults and one child and found that 20 to 60 per cent of the phytin was excreted unchanged in the faeces. It would appear from these investigations that phytin remains largely unchanged in the case of rats, dogs, men and cattle. In the case of pig, however, Rather [1918] found after feeding large amount of phytin in the natural state that almost all the phosphorus was excreted in the form of orthophosphoric acid and his conclusion was that pigs can hydrolyse the phytin completely. Lowe and Steenbock [1936] also fed rats

with prepared phytin with basal ration and they found that phytin as a source of phosphorus was without significant value but equivalent amounts of phosphorus given as phosphoric acid "resulted in a pronounced improvement in calcification" and "Sodium glycerophosphate was still more beneficial". They, therefore, express that "phytin proved itself to be a poorly available source of phosphorus........".

These factors appear to be chiefly responsible for the poor assimilation and negative phosphate balance in the case of D_5, D_8 and D_4 in Experiment B in spite of an ingestion up to the range of 43 grms. P_2O_5. Possibly at about this stage the inorganic portion of P_2O_5 reached the level of requirement. In any case the behaviour of rice *kura* requires to be studied in greater detail and it is proposed to take it up as soon as possible.

Magnesia

In these experiments the MgO intake has varied from 10 grms. to 33 grms. in the different combinations. The heaviest ingestion has been in the case of the Guinea grass group under Experiment F and the rice *kura* group under Experiment B. The rôle played by magnesia is of much less importance as compared to lime and phosphate. According to Green [1935] the dietary requirements of magnesium are very low and are covered by all ordinary rations. He expresses doubt whether a magnesium deficiency disease would ever be found to occur naturally in farm animals. From the results of these experiments it would appear that the general tendency of positive balance is manifest from the stage when the intake has been about 15 grms. and upwards. The results under *aman* straw and cake (Experiment C) suggests that when the intake is sufficiently low to record a negative balance it seems to react upon the economy of soda balance which has been uniformly negative in those very instances under Experiment C in which MgO was deficient. The soda ingestion here was about 17 grms., and with the same quantity of ingestions under Experiment B where MgO ingestion was high, seven out of nine have recorded a positive balance. In experiment E the soda ingestion was similar but MgO was moderate and here both recorded positive balances. There is thus some tendency of inter-relation which is chiefly noticeable if MgO ingestion falls below 12 grms.

Amongst the feeds tested rice *kura* contains the largest quantity of MgO (about 2·6 per cent on dry matter). It has been found by Mendal and Benedict [1909], Hart and Steenbock [1913] and Bogert and McKittrick [1922] that excessive intake of Magnesia gives rise to an increased elimination of calcium. Henry and Morrison [1928] state that those feeds which contain much magnesium in proportion to calcium, such as wheat bran and middlings, when given in excessive amount for long periods "are said to cause a weakening of the bones, leading to such troubles as *bran disease* or *miller's horse ricket*". In rice bran there is much magnesium in proportions to lime and this might be another aggravating cause (in addition to those dealt under lime and phosphorus) to bring such poor result.

Potash

There is generally an appreciable amount of potassium in all plant materials commonly used and so normally there is no great likelihood of a natural shortage. In rice straw potash is present to the amount of 1·6 per cent to 2 per cent on dry matter and is about 5 to 10 times as much as Na_2O. In the experiments under reference the ingestion of K_2O varied from 53 grms. to 89 grms. per 500-lb. live-weight in the combinations in which paddy straw formed the main roughage (Experiments A to E). It will be noted that although the ingestions of K_2O were quite large, a very large amount found its way out specially through the urine so that a positive balance seems to be controlled by a certain level of ingestion which in these experiments was about 66 grms. per 500-lb. live-weight.

In the experiments conducted by Warth [1926] the feeding was divided into two groups, *viz.*, rice straw and hay, and the animals were of three live-weights, *viz.*, 1,000-lb., 750-lb. and 500-lb. The ingestion of potash in his 500-lb. group animals varied from 63·4 grms. to 92·5 grms. in the rice straw group, as compared to 25·6 grms. to 31·2 grms. in the hay group. Unfortunately he has not given the complete balance picture, but as urinary excretion embraces the major part of potash it gives an indication of the balance specially if it exceeds the intake. It is seen from his data * that in the case of the paddy straw feeders two out of three under 750-lb. live-weight and one out of three under 500-lb. have records in which the urinary excretion of potash has exceeded the intake, thereby leaving no doubt of the tendency of a negative balance despite large ingestion. In the case of hay eaters, however, no such inference is possible as the urinary excretion was lower than the intake.

As the behaviour of straw eaters is one of the main objects of comparison it will be noted that in spite of limited data there is some parallelism with the Bengal figures. In the latter the positive balance is maintained when the ingestions generally exceeded 66 grms. per 500-lb. live-weight. In Warth's experiments the one recording negative balance under this live-weight had an ingestion equivalent to 58 grms. and 78 grms. on the basis of 500-lb. computation (the actual ingestions being 84·2 grms. and 117·9 grms. of intake). Both indicate that there is a certain level below which positive balance becomes uncertain.

It should be stated, however, that whether in the case of positive or negative balance the very large excretion is as much an evidence of low economy as it is an indication of the ingestion exceeding much about the requirement. Yet since rice straw forms the main part of roughage there is no way of avoiding the large ingestion of potash through it.

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*Table XVI, page 54 of the *Memoris of the Dept. of Agri. in India* (Chemical series), Vol. IX, No. 2.

The question which opens out an interesting field of study is whether such a large ingestion of potash is associated with any deleterious effect. The literature on the subject is rather conflicting.

Theiler, Green and du Toit [1927] have given an account of their feeding experiment in which "higher potassium ration" containing 44 grms. K_2O and 2 grms. Na_2O was administered. The K_2O was later on increased to 64 grms. The weights of the two animals were 470 lb. and 450 lb. at the start and increased to 1,000 and 1,130 lb. in about two years. The authors remark that on "increasing the total K_2O to 64 grms. (ratio 32 to 1) Heifer 878 died within a few days with symptoms of tymphanitis, but showed no characteristic post-mortem lesions upon which to base a definite diagnosis". Its companion became ill, lost appetite and declined steadily in weight for two months, recovered and gave birth to a weak small calf which died five days later while the mother suffered from "severe mastitis, following retention of the after-birth, did not respond to treatment and was killed in extremis". The authors are, however, inclined to believe that "no disease has been manifested" and that "so far as growth is concerned a relatively high proportion of potassium to sodium appear to have little influence". Green [1935] in a more recent communication adheres to the views of his associates and states that "no naturally occurring disease has been proved to be due to deficiency or imbalance between them". On the other hand Orr [1925] considers that "the ratio of sodium to potassium affects the assimilation of both calcium and phosphorus". The behaviour of lime assimilation as exhibited in the present experiment (*vide* balance figures) would rather seem to support the latter statement.

At the same time the ingestion of potash under the present experiment was considerably higher than those of Theiler, Green and du Toit [1927]. In their case the maximum ingestion was 64 grms. per 1,130-lb. or 28·3 grms. per 500-lb. as compared to 53 to 89 grms. in the paddy straw feeding. As the balance figures have shown there has been a higher lime requirement under this feed. It is, therefore, very likely that the assimilation of lime is probably closely related to the quantity of potash. Hawk [1931] remarks that potassium has an important relation with calcium in maintaining the normal rhythm of contraction and relaxation of heart muscles. The classical experiment of Ringer [1880-82] on the perfused heart show that minute changes in the concentration of calcium or potassium in the perfusing fluids have a profound effect on the activity of the heart. A deficiency of potassium will prevent the heart muscles from relaxing and an excess will relax so completely that it stops beating. Remi and Müller's [1931] experiment with respect to rachitic symptoms associated with high potash intake has been already referred to under lime. The experiments of Richards [1927] show that the addition of potassium salts to a ration of cereal in the case of growing pigs leads to a decreased retention of nitrogen, phosphorus and calcium.

As the large potash ingestion under Bengal condition is unavoidable, attention should be directed towards a better adjustment of other minerals specially lime and phosphate. It need hardly be stated that the animals in Bengal have been under a straw feed for generations and for good or evil they have been accustomed to it. Possibly they have acquired some amount of tolerence and immunity from the deleterious effect, if any, which the larger ingestion might occasion.

Effect of potash on urination

There is another point which also merits attention. Warth [1926] found in his experiments that " rice straw induces a marked and persistent diuresis " and his conclusion is that " the diuresis is intimately related to potash excretion ". Under Bengal conditions, however, this sort of diuresis has not been noticeable.

TABLE VIII

Feed	Urine voided grms.	Water consumed grms.
Aman straw only (Experiment A)	2658·2	8471·0
Aman straw and rice *Kura* (Experiment B) .	3412·8	12853·0
Aman straw—1-lb. linseed cake (Experiment C) .	2663·2	13674·0
Aman straw—1-lb. cake	3492·9	14106·8
Aman straw—2-lb. cake	3917·8	15640·5
Aman straw—3-lb. cake	4196·9	17028·5
Aus straw only (Experiment D) . . .	2585·5	11095·0
Aus straw—linseed cake (Experiment E) . .	3788·9	17191·0
Hyacinth group (Experiment F)	3987·3	3898·0
Hyacinth group with 1-lb. cake	8802·6	7834·0
Napier group (Experiment F)	10169·3	5234·0
Guinea grass group (Experiment F) . .	6326·6	4762·0

In fact, as will be noted from Table VIII, when rice straw was fed alone the urination was lowest. These data represent actual averages and have not been converted to any uniform live-weights, but as the animals were more or less similar,

they give a fairly correct indication of the behaviour of urine excretion under the different feeds. It should be noted that Hyacinth has the largest percentage of potash and the ingestion of K_2O was only next to that of Napier grass but even here the urination did not show a perceptible rise until it was supplemented with cake. Similarly, when straw was supplemented with cake, the urination was higher. Thus, so far as paddy straw is concerned, no marked diuresis could be ascribed to it under Bengal conditions. Ramiah's work at Coimbatore also points to same conclusion [Ramiah, 1932]. On the other hand the addition of cake seems to induce more diuresis.

The only exception has been in the case of Napier group. Here potash ingestion was heaviest and urination was also the highest. This might be suggestive of a partial association of potash with diuresis but as will be apparent from the above results there might be other factor or factors. Warth also states that " while the amount of urine is mainly dependent upon potash there is another subsidiary factor at work ", but according to him " its nature has not been established yet ".

It remains only to be stated that when potash ingestion is heavy, as typified in the case of the Hyacinth and Napier groups under Experiment F, the retention capacity of potash is lowered as is exemplified in the wholesale negative balances under Hyacinth and one out of two under Napier. Possibly it also induces a craving for more sodium chloride as is suggested by Bunge's classical experiment. This part has been further discussed under sodium chloride.

The behaviour of the Guinea grass group is interesting from another standpoint. This group had a little over 72 to 74 grms. of potash, which would seem to be sufficient but both the animals have recorded a negative balance. But here lime ingestion was very high and it cannot be said whether this was responsible for an adverse effect.

Briefly the results indicate that—

(1) with rice straw feeding the potash requirement is probably about 66 grms. minimum per 500-lb. live-weight. Possibly about 70 to 80 grms. should be enough.

(2) In the case where potash is heavily ingested as in Hyacinth and Napier groups, it induces an excretion greater than the intake.

(3) Where lime ingestion is very high, as in the case of Guinea grass, the amount of potash ingested (72 to 74 grms.) did not record a positive balance.

(4) Rice straw did not induce diuresis as was noticed by Warth at Bangalore.

Sodium and chlorine

These two minerals are taken together because sodium chloride is the most convenient form in which they are supplied to the animal. Sodium chloride also forms a component part of the body fluid. Sodium salts exert a specific influence upon the irritability of the nerves and the relaxation of muscles. Overton [1904] showed that frog muscle immersed in isotonic cane sugar lost its excitability and that restoration could be brought about by a sodium salt or in a less degree by a lithium salt but not by salts of potassium. For the normal contraction of the heart muscle a balance must exist between the calcium and sodium contents of the blood [Hawk and Bergeim, 1931].

The above facts by themselves would induce a natural demand for common salt by the animal, and possibly this demand is further increased on account of the presence of a large amount of potash salts in plant material. This has been suggested by a highly interesting experiment by Bunge [1873]. He attempted to attribute the craving which herbivorous animals have for common salt to an excess of potassium in the diet. According to him when a salt such as potassium citrate gains entrance into the blood stream, a proportion of it reacts with the sodium chloride forming potassium chloride and sodium citrate. As the kidney functions in keeping the composition of the blood nearly constant, the abnormal constituents sodium citrate and excess of potassium salts are eliminated. In this way a high potassium salt intake may cause increased elimination of sodium and chlorine. This is partly indicated in the balance figures of the Hyacinth and Napier groups under Experiment F in which potash ingestion was very heavy. One of the sodium figures out of two and both the chlorine under Hyacinth are negative, while in the case of Napier one chlorine out of two has been negative. Sodium figures were, however, positive.

At the same time, it should be stated that experiments on heifers carried out by Hart, McCollum, Steenbock and Humphry [1911] show the incompleteness of Bunge's theory. These authors have different groups of animals with rations of different cereals and all the animals had free access to salt. They found that the sodium chloride consumed had no relation to the amount of potassium in the ration, either as such or when supplemented with potassium salt. Godden's work in conjunction with Richards and Husband [1928] also shows similar conclusion. Miller's later investigation on pigs [Miller, 1922] shows that a sudden increase of potassium salts in the diet caused an immediate increase in sodium and chlorine excretion during the following twenty-four hours period but as stated by Bunge, the excretion of sodium and chlorine in Miller's experiment also decreased when the high potassium intake was continued and Miller says that "the animal can distribute its sodium and chlorine as required by the different part of the body during the absorption of large quantities of potash, which may at other times cause increased sodium and chlorine excretion".

Turning now to the other experiment, *viz.*, to those in which rice straw forms the main roughage, it will be noted that in Experiments A, B, D and E, sodium has exhibited a general tendency of positive balance. In these the amount of intake varied from 14 to 23·8 grms. and would seem to be about the requirement. But although this might generally be so the results under Experiment C suggest that in the event of a deficiency in magnesia the soda balance is likely to be adversely affected. Here the soda intake was about 17 grms., but three out of four have recorded a negative balance and it is these three whose magnesia balances are also negative. There are also probably other factors—for instance the lime supply was just on the border line and inclined towards deficiency as is noticeable from the two negative balances out of four. The chlorine ingestion was also very high—in fact the highest with the exception of the Napier group. Leaving these (under Experiment C) the weight of evidence from the majority of other experiments is in favour of an amount of sodium equivalent to 16 to 18 grms. Na_2O per 500-lb. live-weight or 32 to 36 grms. per 1,000-lb. live-weight. The amount would correspond to 30·17 to 33·94 grms. NaCl per 500-lb. and 60·34 to 61·88 grms. per 1,000-lb. In actual fact about half the amount is supplied through the feed. This should not mean that the whole of it should be supplied as NaCl.

With respect to chlorine the ingestion has varied from about 17 to nearly 30 grms. The heaviest ingestion has been in the case of Napier group (Experiment F) closely followed by *aman* straw and cake under Experiment C. It cannot be stated whether such an amount as was ingested in these two experiments was really excessive or not. McCollum, Simmonds and Becker [1922] noticed an inflammatory condition of the eyes resembling xerophthalmia which he calls salt opthalmias due to excess of chlorine in the food. No such condition was noted in the present experiment though the Hyacinth animals were distinctly below condition but in this as already stated there was deficiency of other nutrients also. In general, it would seem that an ingestion of about 20 grms. of chlorine should ordinarily meet the necessary requirement for animals of 500-lb. live-weight. But Experiment B under *aman* straw and Rice *kura*, and the Hyacinth group under Experiment F are exceptions to this. In these two, negative balances have been the conspicuous feature. In both cases there was some departure from normal conditions in as much as, in Experiment B, the intake of calcium (as indicated from the other experiments with rice straw) was very low and phosphate very high, while in the Hyacinth group calcium and potash were very high and phosphate and nitrogen low. Leaving these, the requirements of chlorine in all ordinary cases under rice straw would appear to be satisfied as already stated with an amount approximating to 20 grms. per 500-lb., although in some experiments larger amounts have been ingested. This would correspond to 32·97 grms. NaCl and about half of it is expected to be provided through the feed.

It need hardly be stated that sodium and chlorine assume their importance both from their indispensable nature in the system as well as from their instinctive demand as salt by all animals including human beings. Wild herbivorous animals have been known to travel great distances in the search for salt licks to satisfy this craving. The question now arises as to what should be the minimum amount which should be fed to the cattle specially in the form of common salt—Kellner [1915] prescribes the daily need of average cow at $\frac{3}{4}$ to $1\frac{1}{2}$ ounce of salt. This works out at 11·3 to 22·6 grms. Na_2O (*i.e.*, 5·6 to 11·3 grms. per 500-lb. live-weight) and 12·93 to 25·85 grms. of chlorine (*i.e.*, 6·47 to 12·94 grms. of chlorine per 500-lb.). He has further stated that in the case of difficultly digestible materials, the quantity may be increased to $2\frac{1}{2}$ ounce (equivalent to 37·66 grms. soda and 48·08 chlorine or 18·83 grms. soda and 21·54 grms. chlorine per 500-lb.) but he emphasises that " more than these quantities *should not under any circumstances be given* ". (The italics are ours.) Buffagni [1935] gives the toxic dose of sodium chloride for pigs as 1 grm. per kg. of body-weight and considers that cattle should not be given more than 60 grms. per day. In some of the recent experiments at Krishnagar (not reported here) about 60 grms. of salt were fed to animals of about 500 to 600-lb. live-weights and no serious trouble was noticed. There was no doubt that some animals were out of condition but this only occurred when they were on a high dose of mustard cake. It seems, however, that in Bengal the ordinary dose of common salt fed to the animals is a little higher than the amounts indicated by Kellner and others. This amount although arising out of the general usage of the country is probably an unconscious adjustment necessitated from a larger ingestion of potash through rice straw feeding.

Relation of minerals to one another, their ratios, etc.

The interpretation of the data on mineral metabolism carries with it some inherent difficulties in as much as there is no means by which the actual amount absorbed or assimilated in the system can be properly ascertained. This has led various investigators to adopt more or less empirical procedures which can at best serve as rough guides. In fact, as Orr [1925] states " it is impossible in our present state of knowledge to do more than make an empirical adjustment by adding inorganic salts containing elements thought to be deficient in the rations ". In another place [Orr, 1929] he suggests that as the amount of food needed by the animal is determined by its energy requirement the truest basis of comparison is the energy unit. He has proceeded in this way and based his calculations on energy values, on the assumption that "40 per cent of the ether extract is digestible fat and the total nitrogen-free extractives are equal to the sum of the digestible nitrogen-free extractives and the digestible fibre ". Although his values were not evidently derived from direct experiments yet the conception originated from the estimation of digestibility coefficients and are presumably meant to be equivalent to the total digestible nutrients expressed as calories.

In this way he has tried to draw a comparison of minerals per 1,000 calories. Theiler, Green and du Toit [1927] are also inclined to a similar opinion, as they stressed that it was not the percentage of phosphorus on dry matter which mattered but the amount in relation to the energy value of pasture. In a more recent publication Theiler and Green [1932] have also suggested that as a rough guide "a round figure of 1 per 100 starch equivalent" may be taken as safe for either P_2O_5 or CaO throughout the bovine or ovine life. Marek [1924] has emphasised the importance of "Alkali - Alkalizität and Eldarkali - Alkalizat". Wellmann [1931] working in co-operation with Marek states that the relationship of the alkaline earths calcium and magnesium and their ratio of phosphoric acid are important. This relationship (E. A.) is calculated on the basis of 100 grms. dry matter as follows—

$$\text{E. A.} = \frac{(\text{CaO mg. equiv.} + \text{MgO mg. equiv.} - P_2O_5 \text{ mg. equiv.}) \times 100}{\text{Grm. dry matter}}.$$

Theiler, Green and du Toit [1927] have, however, strongly attacked what they call the "fallacy of Marek", and in the course of their discussion have remarked that the data at present available "are too fragmentary to justify any generalisation". These diverse views only emphasise the complexity of the whole subject and any attempt towards interpretation of the results on these diverse lines can only be made both with caution and reservation.

There is no doubt that the results need scrutiny from all standpoints and in order to facilitate the same the best course would appear to be to assemble all the relevant data in the different forms so that they might embody a collection for future interpretation in the light of more suggestive information which may accumulate in course of time. Accordingly, the data have been worked out in the attached Appendices on the basis of different ratios, calorific relations (Orr's), Marek's E. A. values and so forth. It should, however, be stated that the calories here have been worked out from actual digestibility values and thus involves no assumption as was unavoidable in Orr's figures.

Although the interpretation on the basis of these factors is withheld for the present it should be stated that whatever might be the relation of mineral matter either with respect to the absolute amount or with respect to their proportions to each other the nature of the feed is by far the chief determining factor. There is no doubt that the animal has what may be called a minimum requirement of minerals but the criterion for this minimum is not always a phase indicated by the absolute minimum need of the animal or of the presence of this absolute minimum in the feed but is a condition mostly regulated by the nature of the feed. In other words, with different feeds this minimum is likely to vary. This is more strikingly illustrated in the lime requirement under rice straw as the main roughage. It has already been stated that according to Kellner an amount of lime equivalent to 22·7 grms. CaO per 500-lb. would "sufficiently meet all

requirements ". Similar results have been obtained by other workers also, but the results under paddy straw feeding show that instead of this amount being sufficient it is below the minimum, since positive balance could not be attained until the intake was at least 24 grms. CaO. This aspect is thus entirely controlled by the nature of the feed.

Another feature that may be mentioned is that under Bengal conditions, although there is some deficiency of CaO, the deficiency of P_2O_5 is more definite and possibly it is the limiting factor in the large majority of cases. For the present the best practical course would be to see that the minimum supply is ensured. Like Theiler, Green and du Toit [1927] the present authors believe that the basic ratio only becomes important " when the preponderance of one over the other becomes so great that the liberal physiological mechanism for preservation of neutrality of the tissues are strained " and they are inclined to the view as expressed by Theiler and Green in a later publication [1932] that " attention may be concentrated upon the absolute intake of calcium and phosphorus per day except where the imbalance is very wide ".

SUMMARY

1. In a fairly comprehensive series of experiments arranged under six broad combinations embracing thirty individual data, an intensive study of the mineral requirements of the animals have been attempted. In five of these combinations rice straw (both winter or the *aman* variety and autumn or the *aus* variety) formed the main roughage. The sixth combination consisted of green feeds divided into three sub-groups of Hyacinth, Napier grass and Guinea grass.

The experimental scheme was drawn up in such a way as to conform as far as possible to the type of feeding used in Bengal which is typical of North-East India.

2. It is found that in all combinations except the green feed groups (Experiment F), rice straw has been the chief source of lime, potash, magnesia (except rice-*kura* group. Experiment B), also largely soda and chlorine although they are appreciably supplied through common salt, while phosphate has been mainly derived from concentrates—cake and *kura* (rice bran).

In the green feed combinations, Guinea grass and Hyacinth provided the predominant share of lime and Napier the least. But the latter provided the largest intake of potash closely followed by Hyacinth. The potash content is highest in the Hyacinth but the ingestion of Napier was very heavy and hence the potash intake went up. Phosphate was well supplied through Guinea grass and Napier grass but lowest by Hyacinth.

3. The investigation represents conditions in which some of the important minerals have gone up from the stage of deficiency to that of adequacy, thus suggesting the lowest limit compatible with positive retention. All computations

have been based on the prevailing live-weights (*viz.*, 500-lb.) of the local breeds and on this basis the minimum mineral requirements (when paddy straw froms the main roughage) appear to be 24 grms. CaO, 10 grms. P_2O_5, 15 grms. MgO, 70 grms. K_2O, 17 grms. Na_2O (equivalent to 32·05 grms. NaCl) and 20 grms. Cl_2 (equivalent to 32·97 grms. NaCl). The sodium and chlorine figures show a striking coincidence with the equivalent of NaCl.

4. The lime requirement seems to be higher than in investigations of many others. It is suspected that this might be associated with high potash ingestion through rice straw or the presence of anticalcifying agents in cereals (as found by some investigators) or the combination of both (*vide* discussion in the text). There might be other factors also.

5. The general ingestion of K_2O is quite large under rice straw feeding, but inspite of it positive balance is not maintained until the ingestion is about 66 to 70 grms. per 500-lb. live-weight.

Heavy potash ingestions seem to induce heavier excretion even greater than the intake as will be evident from the behaviour of Hyacinth and Napier. On the other hand, higher ingestion of CaO seems also to upset the potash balance as indicated from Guinea grass.

6. A deficiency of MgO seems to affect the soda balance as is indicated from Experiment C.

7. Rice *kura* (bran) is very poor in CaO and unusually rich in phosphate (over 6 per cent). The CaO and chlorine figures have definitely given negative balance; while in spite of heavy P_2O_5 ingestion (39 to 59 grms.) negative balance has been recorded up to the stage of 43 grms. This is probably due to the presence of phosphorus in the rice bran mainly as phytin which is not readily assimilable. Various workers have noticed the presence of anti-calcifying agents in cereal grains and similar factors might also be further aggravating causes. Rice bran should, therefore, be supplemented with lime and chlorine on one side, while its phosphorus compound should be brought into assimilable form by hydrolysis.

8. The results indicate that *aus* straw is considerably superior to *aman* straw in minerals and protein and hence in general feeding values, but both straws are poor in phosphate and slightly deficient in lime. The phosphate might be conveniently supplied through cake while a small supplement of chalk would make up lime deficiency.

9. In the case of green feeds, Guinea grass is better balanced than Napier and Hyacinth, but the feeding should be regulated in order to avoid a large ingestion of lime. Napier should be supplemented with $CaCO_3$ and both Napier and Hyacinth should be so regulated as not to involve a large ingestion of potash through them. They both (specially Hyacinth) require some cake supplement. Hyacinth is not a fodder but when there is a fodder scarcity it is often used as such in some parts of Bengal, and hence formed a subject of investigation.

10. No interpretation has been attempted on the basis of ratios, the bearing of one mineral to the other, the E. A. values and so forth, as it is felt that information on these aspects is still meagre. An intermediate course has, therefore, been adopted by making a collection of all relevant data, ratios, etc., in the Appendices in order that they might be available for interpretation in the light of future development.

It need hardly be stated that whatever might be the relation of mineral matter either with respect to the absolute amount or to their respective proportions, the nature of the feed is by far the chief determining factor, and although there is some deficiency of lime in rice straw feed the deficiency of phosphorus is probably the chief limiting factor.

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APPENDIX I

Composition of different feeds (on dry basis)

Feed	Organic matter	Crude protein	Ether extract	Crude fibre	Nitrogen free extract	Ash	Silica	Soluble ash	Nutritive ratio 1 :	Total digestible nutrients per 100 lb.	Starch equivalent per 100 lb.
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent			
Aman paddy straw . .	85·309	2·894	0·868	34·215	47·332	14·691	10·821	3·870	*	29·9	10·0
Aman paddy straw . .	85·785	3·811	0·964	34·221	46·789	14·214	10·250	3·965	116	44·17	24·18
Aman paddy straw . .	86·161	3·200	1·191	33·763	48·007	13·839	10·190	3·649	56	44·40	24·34
Aman paddy straw . .	86·786	3·750	1·147	33·239	48·650	13·214	9·082	4·132	47	44·40	24·65
Aus paddy straw . .	88·796	5·879	1·713	33·425	48·779	11·204	7·372	3·832	23	43·32	24·25
Guinea grass . . .	86·056	7·969	1·733	32·360	43·995	13·944	8·066	5·878	10·3, 10·9	51·7	28·9
Napier grass . .	83·297	5·349	1·894	31·900	44·109	16·703	6·632	10·071	20·3, 25·0	48·6	38·6
Water hyacinth . . .	83·533	6·544	1·700	24·696	50·593	16·467	0·404	16·063	*	34·3	28·0
Linseed cake . . .	89·611	31·606	6·812	9·614	41·579	10·389	3·335	7·054	1·5	67·41	65·20
Linseed cake . . .	89·871	30·331	6·327	9·346	43·867	10·129	3·274	6·855	1·6	66·69	64·45
Rice Bran (*kura*) . .	83·888	15·969	20·321	10·821	36·777	16·112	4·678	11·434	8·2	57·13	43·99
Rice Bran (*kura*) . .	84·440	12·000	20·455	17·441	34·541	15·560	4·414	11·146	11·4	57·71	44·54

* The nitrogen digestion was negative.

APPENDIX II

Mineral composition and ratios of different feeds

Feed	Composition (on dry matter)							Ratio				
	CaO	MgO	K_2O	Na_2O	P_2O_5	Cl_2	N	$CaO : P_2O_5$	CaO : MgO	$CaO : K_2O$	$K_2O : Na_2O$	E. A.
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent					
Aman rice straw	0·401	0·290	1·575	0·354	0·139	...	0·463	2·89	1·35	0·26	4·45	22·82
Aman rice straw	0·463	0·200	1·904	0·203	0·123	0·441	0·610	3·76	2·32	0·24	9·38	21·24
Aman rice straw	0·484	0·353	1·820	0·270	0·100	...	0·512	4·84	1·37	0·27	6·74	30·55
Aman rice straw	0·500	0·365	1·838	0·261	0·108	...	0·600	4·63	1·37	0·27	7·04	30·38
Aus rice straw	0·635	0·400	2·032	0·207	0·176	0·336	0·941	3·61	1·59	0·31	9·82	57·21
Guinea grass:	1·232	0·728	1·451	0·572	0·541	0·135	1·275	2·28	1·69	0·85	2·54	0·99
Napier grass	0·461	0·373	4·801	0·297	0·804	0·571	0·863	0·57	1·24	0·096	16·17	154·32
Water hyacinth	3·423	0·957	5·913	0·186	0·361	3·484	1·047	9·48	3·58	0·58	31·79	—34·54
Linseed cake	0·480	0·951	2·100	0·473	2·340	0·048	5·057	0·21	0·51	0·23	4·44	—13·07
Linseed cake	0·652	1·009	1·751	0·567	2·045	0·061	4·853	0·32	0·65	0·37	3·09	—125·63
Rice bran (*kura*)	0·206	2·641	1·946	0·197	6·250	0·019	2·555	0·03	0·08	0·11	9·88	—123·92
Rice bran (*kura*)	0·242	2·607	1·780	0·467	6·200	0·029	1·920	0·04	0·09	0·14	3·82	0·37

APPENDIX III

Showing distribution of minerals per 1,000 calories in different feeds

Feed	CaO grms.	MgO grms.	K_2O grms.	Na_2O grms.	P_2O_5 grms.	Cl_2 grms.	Protein grms.
Aman straw	3·600	2·600	14·700	3·250	1·200	...	26·106
Aman straw	2·787	1·204	11·464	1·222	0·74	...	16·593
Aman straw	2·899	2·114	10·902	1·617	0·599	..	19·162
Aman straw	2·090	2·186	11·009	1·563	0·646	...	22·462
Aus straw	3·898	2·455	12·475	1·270	1·080	2·062	36·106
Guinea grass	6·300	3·700	7·400	2·900	2·800	0·935	41·047
Napier grass	2·500	2·000	25·000	2·600	4·400	3·000	29·600
Water hyacinth	28·900	8·000	50·000	1·500	3·000	20·000	55·363
Linseed cake	18·938	37·521	82·854	18·662	92·323	1·893	124·700
Linseed cake	25·998	40·233	69·820	22·614	81·544	2·432	120·962
Rice bran (*kura*)	0·959	12·294	9·059	0·917	29·096	0·088	74·337
Rice bran (*kura*)	1·115	12·014	8·203	2·152	28·573	0·133	55·300
* Cultivated pasture	3·700	...	23·080	1·850	2·700	3·520	65·300
* Cow's milk	3·420	...	6·400	1·560	3·470	1·390	51·600

* Orr's figures taken from the British association address but converted into the present form.

Appendix IV

Intake of dry matter, ratios of minerals and E. A., etc.

(Calculated on 500-lb. live-weight.)

Experiment No.	Animal No.	Dry matter consumed *	Total digestible Nutrients * lb.	Starch Equivalent. * lb.	$CaO : P_2O_5$	$CaO : MgO$	$CaO : K_2O$	$K_2O : Na_2O$	E. A.	Nutritive ratio 1:	Actual live-weight lb.	Loss or gain in live-weight lb.
Aman straw only A.	D_7	3667·6	1·952	0·827	2·885	1·383	0·255	2·426	22·817	↑	343	—30·880
	D_8	3360·1	2·028	0·558	2·885	1·383	0·255	2·303	22·815	↑	335	—30·431
Aman straw and Rice *Kura* B.	D_1	4064·8	4·015	2·464	0·406	0·611	0·240	4·409	5·652	24·96	550	0·585
	D_5	4056·4	4·233	2·420	0·469	0·666	0·244	4·340	9·075	32·44	536	—0·214
	D_7	3587·1	3·691	2·250	0·307	0·511	0·232	4·191	—2·136	24·84	533	—0·335
	D_8	3900·8	3·812	2·338	0·449	0·648	0·243	4·202	8·074	34·34	512	—0·111
	D_9	3825·3	3·939	2·344	0·369	0·577	0·238	5·022	3·211	25·54	612	0·444
	D_2	3278·8	3·743	2·174	0·244	0·441	0·237	3·623	—10·868	22·44	585	—0·232
	D_3	3594·2	3·706	2·295	0·311	0·522	0·245	3·803	—2·220	24·55	568	0·830
	D_4	3545·5	3·999	2·205	0·379	0·592	0·250	3·854	3·655	28·12	570	—0·150
	D_6	3487·4	3·358	2·290	0·259	0·461	0·240	3·738	—8·565	24·35	589	1·115
Aman straw and 1 lb. Linseed cake C.	D_8	4544·6	4·303	2·420	1·517	1·762	0·244	5·022	16·868	19·00	500	—0·607
	D_1	4270·5	4·244	2·504	1·549	1·775	0·243	4·903	17·019	18·00	552	—0·133
	D_4	4542·9	4·613	2·764	1·533	1·769	0·244	5·026	16·995	22·00	509	—0·419
	D_7	4510·2	4·677	2·846	1·479	1·745	0·243	4·997	16·648	17·00	484	—1·932

Aus straw only D.		D_1	3630·9	3·367	1·833	3·635	1·585	0·314	4·991	35·415	23·00	490	0·439
		D_3	3586·4	3·452	1·938	3·632	1·587	0·314	5·212	35·370	22·00	543	—0·442
		D_7	3254·1	3·132	1·753	3·634	1·584	0·314	4·641	35·409	24·00	474	—0·422
Aus straw and Linseed cake E.		D_2	4058·6	4·226	2·585	2·424	1·482	0·317	5·165	33·133	14·00	526	0·361
		D_6	4609·9	4·428	2·559	2·399	1·479	0·317	5·089	33·068	14·00	449	0·702
		D_9	3696·5	3·834	2·333	2·459	1·486	0·317	5·270	33·240	15·00	603	0·580
		D_4	4432·1	4·609	2·657	2·185	1·457	0·317	5·327	33·368	15·00	541	0·499
		D_5	4701·0	4·577	2·663	2·167	1·445	0·351	4·762	33·320	12·00	479	0·282
		D_8	4103·8	3·744	2·074	2·126	1·389	0·335	5·042	32·900	11·00	553	0·434
Aman straw and green feed F.	Hyacinth Group	D_1	2946·5	2·271	1·357	9·023	3·640	0·608	5·669	63·770	↑	350	—2·281
		D_2	2604·6	2·263	1·769	7·338	3·974	0·498	5·471	85·346	↑	376	0·971
	Napier Group	D_4	4409·8	4·810	3·565	0·714	1·260	0·113	7·833	9·484	26·00	448	—0·305
		D_5	3997·3	3·990	3·840	0·726	1·271	0·115	7·416	7·317	33·00	447	—0·857
	Quenin Group	D_3	5023·1	5·707	4·173	2·477	1·798	0·812	2·107	55·095	11·80	469	—1·108
		D_6	4881·7	5·340	3·865	2·299	1·675	0·767	2·123	52·791	12·30	517	0·376

* "Not calculated on power function of live-weight but on direct basis as the object was to note the direct relation with minerals."

↑ The nitrogen digestion was negative.

APPENDIX V

Intakes of minerals per 1,000 calories

Experiment No.	Feeds	Animal No.	CaO grms.	MgO grms.	K_2O grms.	Na_2O grms.	P_2O_5 grms.	Cl_2 grms.	Protein grms.
A.	*Aman* straw as single feed	D_7	4·41	3·19	17·33	7·14	1·52	5·71	31·81
		D_8	3·96	2·81	15·28	0·85	1·34	5·41	28·06
B.	*Aman* straw and Rice *kura*	D_1	2·62	4·30	10·93	2·48	6·48	2·75	30·81
		D_5	2·52	3·79	10·33	2·38	5·37	2·67	27·75
		D_7	2·44	4·77	10·53	2·51	7·96	2·74	33·31
		D_8	2·68	4·13	10·04	2·62	5·97	3·22	30·06
		D_9	2·64	4·32	10·49	2·09	6·76	2·91	30·56
		D_2	2·22	5·04	9·37	2·58	9·12	2·68	30·81
		D_3	2·51	4·82	12·93	2·70	8·08	1·16	31·18
		D_4	2·39	4·01	9·50	2·46	6·27	2·84	27·18
		D_6	2·66	5·78	11·11	2·97	10·27	3·13	35·81
C.	*Aman* straw and Linseed cake	D_3	2·75	1·55	11·33	2·31	1·78	3·58	35·37
		D_1	2·90	1·64	11·90	2·36	1·91	3·67	37·50
		D_4	2·70	1·52	11·09	2·20	1·76	3·43	34·62
		D_7	2·64	1·51	10·87	2·17	1·78	3·36	34·75
D.	*Aus* straw as single feed	D_1	4·04	2·55	12·86	2·57	1·11	3·62	37·62
		D_3	3·89	2·45	12·39	2·37	1·07	3·37	35·87
		D_7	3·89	2·45	12·39	2·67	1·07	3·71	37·06

E.	*Aus* straw and linseed cake . .	D_3	3·60	2·43	11·37	2·20	1·48	2·[illegible]3	28·50
		D_5	3·90	2·64	12·33	2·42	1·62	3·21	43·37
		D_6	3·61	2·43	11·42	2·16	1·47	2·90	39·25
		D_4	3·60	2·48	11·36	2·13	1·65	2·78	41·31
		D[illegible]	3·97	2·74	11·32	2·37	1·83	2·83	45·56
		D_6	4·08	2·95	12·18	2·41	1·92	3·01	48·43
	Aman straw and hyacinth . .	D_1	16·64	4·57	27·37	4·82	1·84	5·03	35·43
		D_2	10·61	3·56	21·32	3·89	1·44	4·01	27·43
F.	*Aman* straw and Napier . .	D_4	2·38	1·88	21·05	2·68	3·34	3·62	25·18
		D_5	2·60	2·04	22·68	3·05	3·58	4·05	27·37
	Aman straw and Guinea grass .	D_3	6·14	3·44	7·57	3·59	2·48	1·85	37·18
		D_6	6·02	3·59	7·85	3·70	2·62	1·86	39·18
Orr's figures.	* Cultivated pasture per cent .	...	3·70	...	23·08	1·85	2·70	3·52	65·30
	* Cows milk per cent . . .	...	2·42	...	6·40	1·56	3·47	1·39	51·60

* Orr's figures taken from British Association address, 1925, but have been converted into present forms.

THE OCCURRENCE OF SPINOSE EAR TICK (*ORNITHODORUS MEGNINI* DUGÈS IN INDIA*

BY

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(With Plates XIII & XIV and one text-figure)

On the 15th January 1936, a consignment of ticks collected post-mortem from inside the ears of an Australian horse that had developed symptoms of incoördination of movement evidently as a result of the infestation, was received at the Mukteswar Institute from Captain J. S. Kingston, R. A. V. C., Officer Commanding, Military Veterinary Hospital, Saugor, Central Provinces. These, on examination, proved to be the nymphs of *Ornithodorus megnini* Dugès, the Spinose Ear Tick of America, the occurrence of which had not been previously recorded in India. A specimen of the tick was also forwarded to Mr. Cecil Warburton, at Cambridge, and he confirmed the identification. Concerning the animal from which these specimens of ticks were recovered, Captain Kingston wrote as follows :

> " The animal in question, Equitation School Horse No. 145, an Australian 7 years, has been at the Equitation School for about 4 months. It could rightly be described as insane. It took sudden frights at the approach of man, started without apparent reason, put individuals in hospital who tried to come near it, and would not allow its head to be touched .... I did a post-mortem examination and found about twelve ticks in each ear. They had perforated both ear drums and laid bare the auricular nerves and infected the petrous temporal bones. They were not attached to the mucous membrane inside the ear, but lived in a cheesy mass which they produced in both ears ".[1]

Subsequent to the receipt of these specimens, three further consignments of the same species of tick were received, two of these being collected, as before, from imported Australian horses and one from an Indian mare foaled and reared

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[1]These observations have since been published by Captain Kingston (*see* list of references).

at the Ahmednagar Stud Farm. Concerning this last, the Veterinary Officer, Ahmednagar Remount Depôt, furnished the following interesting information in a letter dated the 15th September 1936 :—

"The case under consideration is of interest, as it is improbable though not impossible that infection could have been carried from Australian horses to this animal. The Stud Farm where the animal was bred is some three miles from the Australian Remount Depot. The animal has never left this stud and has only been in contact with Australian horses to a very limited degree. The number of Australian horses in this Stud would not exceed 12, nor has any case of these ticks occurring among these horses been found. Examinations to date have revealed the presence of Spinose Ear Tick in one other Indian bred horse in the Stud".

It was, however, mentioned by him that the Spinose Ear Tick occurred commonly amongst Australian horses, some eight or nine cases having been found during the preceding two years when the average yearly strength of the Depôt was 750.

As the species seemed to be of more than sporadic occurrence in India, communication was at once entered into with the Director of Veterinary Services, Army Veterinary Department, with a view to obtaining some indication of the extent to which this parasite had established itself as a pest of domesticated animals in this country. At our request, instructions were kindly issued by him to the Veterinary Officers in command of the different stations under him to carry out, as opportunity occurred, an examination of the ears of horses and cattle in their charge for the presence of these ticks. This examination, which has now been completed, has revealed the existence of the Spinose Ear Tick in three stations, namely, Saugor, Mhow and Ahmednagar. The details are as follows :—

(1) *At Saugor.*—Out of 523 animals examined, sixty-eight (or 13 per cent) were found infected, and out of the latter, about 50 per cent were of Australian and an equal number of Indian breed. The infected animals did not "display any definite symptoms, their condition remaining good. Some resented handling of heads and ears and a few were excitable, while some had discharges from the affected ears".

(2) *At Mhow.*—Out of fifty-five horses examined, four (or 7·3 per cent) were found infected, but none of these showed any untoward symptoms. Specimens of ticks were also taken from the ears of a private charger that had developed a violent shaking of the head as a result of the infestation.

(3) *At Ahmednagar.*—Out of 250 Australian horses examined during January to March 1927, twenty-two (or 8·8 per cent) were found infested, the maximum number of ticks found in any one ear being forty-eight. None of the affected animals developed any clinical symptoms.

As to the manner in which *O. megnini* came to be introduced into India, three possibilities suggest themselves : that it may have been brought (1) with mules coming from America ; (2) with stock coming from South Africa, where the tick was first seen in 1898 [Bedford, 1917] ; or (3) with horses imported from Australia. It would, however, appear from a communication, dated the 13th November 1936, received from the Chief of Division of Animal Health, Australia, that the parasite is quite unknown in that country, so that it may be reasonably concluded that it has been introduced with stock coming from one or both of the other two countries mentioned above. This conclusion would seem to find support from a statement, which we have received through the courtesy of the Director of Veterinary Services, Army Veterinary Department, and which shows that the number of American and South African mules received and dealt with by the Ahmednagar Remount Depôt during the past thirteen years was eighty-two and 729 respectively.

From the foregoing, it would appear that the parasite is fairly widespread in this country, and in view of its importance as a pest of domesticated animals, it would seem desirable that veterinary workers in India were made acquainted with the habits and the general features of this tick and also with the symptoms known to be produced by the infestation.

It is my pleasant duty to express my grateful thanks to Mr. F. Ware, C.I.E., F.R.C.V.S., F.N.I., I.V.S., Director of the Imperial Veterinary Research Institute, Mukteswar, for the keen interest with which he has watched the progress of this investigation. In feeding the ticks on horses, I have received much help from Mr. J. A. Idnani, Veterinary Inspector, for which I am deeply indebted to him. My thanks are due to Mr. Ahmed Bakhsh, Artist, for the illustrations, which he has drawn in original from specimens bred out in the laboratory.

Distribution

It is generally agreed that the original home of the Spinose Ear Tick is the United States of America, where it is particularly prevalent in certain south-western semi-arid sections. According to Imes [1918], the climatic conditions in parts of Texas, Oklahoma, New Mexico, Arizona and California are favourable for the rapid multiplication of these ticks. Nuttall and his collaborators [1908] quote Dugès [1883] as having first described the tick from Mexico, where it is reported to be abundant in the State of Guanjuato. The tick is also known to occur in South Africa, where, according to Bedford [1917], it was probably introduced with stock coming from America. Although it is not known when the tick was actually introduced into that locality, Bedford mentions Theiler as having found it at Vryburg in 1912 and he further refers to Manley as having first seen this species in Cape Colony in 1898.

Habits of the ticks and their effects upon their hosts

As the name Spinose Ear Tick implies, *O. megnini* is characterized by the possession of spines on its body in its nymphal stage and it has the peculiar habit of locating itself deep inside the ears of its hosts, which include cattle, horses, mules, sheep, goats, dogs, rabbits and man. Story [1920] states that the tick occasionally feeds on the skin in the case of sheep. In cases of gross infection, the ears may be packed full of these parasites, which frequently crawl into the ear-canal and even attack the ear-drum. According to Pierce [1921], the ears of infected animals are droopy, hair rough, fattening is difficult, and under range conditions, death is not infrequent. In horses and mules marked shyness is observed, and " this is sometimes so extreme that it is almost impossible to halter or bridle an infested animal ". The symptoms are graphically described by Imes [1918] as follows :

" The infested animal usually shakes its head and repeatedly turns it from side to side, meanwhile inverting first one ear and then the other. When irritation and itching are more intense on one side, the animal often turns its head so that the more seriously affected ear is held inverted. There is a tendency to run and scratch the ears, and young animals often run as though endeavouring to relieve the nervous tension. Horses and dogs seem to be more sensitive than cattle to the pain and irritation. They scratch and rub their ears, shake their heads, and often lie down and roll, rubbing their ears on the ground ".

Story [1920] states that young calves suffer most from this pest, its bite producing swelling and abscesses in these animals.

Description of the different stages

The morphological features of the different stage of *O. megnini* have been fully described by Nuttall and Warburton [1908] and a brief and useful description of these stages has also been given by Bedford [1912]. In the description that follows, only such features of the morphology of the tick will be dealt with as are likely to be of use to the veterinary worker in recognizing the parasite under field conditions in India.

Egg (*Plate XIII, fig. 1*).—The egg is circular in shape and measuring about 0·3 mm. in diameter. Like the eggs of other species of ticks, it is dark-brown in colour, which does not appear to alter in intensity during the incubation period.

Larva (*Plate XIII, fig. 2*).—Elongate oval and white in colour, with a long terminal capitulum and with the integument finely striated and provided with bristle-like hairs, Palpi slightly longer than the capitulum, hypostome armed and the digits of chelicerae fairly conspicuous. Legs long and pulvilliform. When unfed, the larva measures about 0·75 mm ; when engorged, it is egg-shaped and measures 2 to 4 mm. in length.

DIFFERENT STAGES OF THE SPINOSE EAR TICK

Ornithodorus megnini

FIG. 1. Egg. ×67.

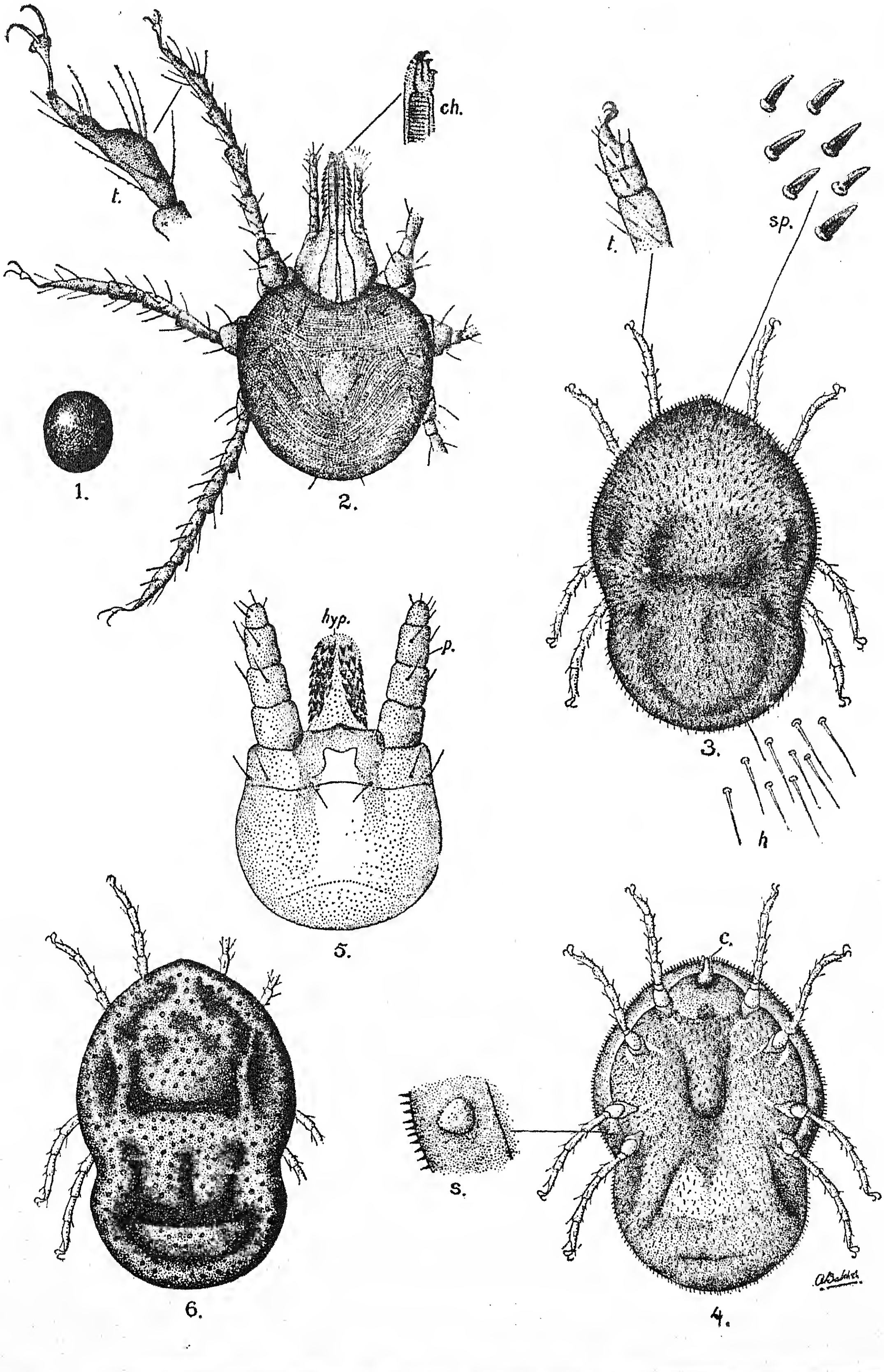
FIG. 2. Larva, unfed, dorsal view. ×67.
ch., distal extremity of chelicera, enlarged ;
t., tarsus I in profile, enlarged.

FIG. 3. Nymph, dorsal view. ×6.
h., bristly hairs, enlarged ; *sp.*, spines, enlarged ;
t., tarsus I in profile, enlarged.

FIG. 4. Nymph, ventral view. ×6.
c., capitulum ; *s.*, spiracle.

FIG. 5. Nymph, mouth-parts (chelicerae omitted). ×67.
hyp., hypostome ; *p.*, palp.

FIG. 6. Adult, dorsal view. × 6.



t.
ch.
1.
2.
t.
sp.
3.
h
hyp.
p.
5.
c.
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6.
4.

Nymph (*Plate XIII, figs. 3, 4 and 5*).—The most characteristic feature of the nymph is that its body is beset with a number of posteriorly-directed spines, from which, as already mentioned, the Spinose Ear Tick derives its common name. These under high magnification appear horn-like with a ringed base and are present both on the dorsal and ventral surfaces and also on the sides of the body, which is conspicuously constricted behind the hind legs. In addition to the spines, there are a number of much smaller bristly hairs covering the whole skin of the nymph. Capitulum sub-terminal or slightly projecting anteriorly, hypostome armed and palpi thick. Genital aperture absent. The spiracles are characteristic, being situated on tubercles between coxae III and IV, somewhat ventral to the dorso-lateral border. Legs fairly stout, with well-developed terminal claws. Colour of engorged nymph dark-brown. The measurements of four replete specimens were as follows: 8·5 × 5·5 mm.; 10 × 6·5 mm.; 8·5 × 5·5 mm.; and 7·5 × 4·5 mm.

Adult (*Plate XIII, fig. 6*).—Body fiddle-shaped, slightly attenuated anteriorly, but rounded posteriorly and sharply constricted behind legs IV. Unlike the nymph, the integument of the adult is provided with small, shallow, circular pits, whilst its dorsal surface is with at least four pairs of symmetrical depressions. Capitulum short and hypostome unarmed, so that the adult is incapable of feeding. Eyes absent. Spiracles circular. Anus situated centrally in a depression posterior to Legs IV. Genital aperture between first pair of coxae. Legs pale-yellow, rather thin, each tarsus being provided with a dorsal protuberance. Colour brown or slate. Size 8 × 5 mm.

Life-history

The female ticks lay their eggs in cracks and crevices of buildings, fences and trees. According to Story [1920], the favourite hiding place of adults is loose, dry manure between stones at the bottom of kraal wall. On hatching out from the eggs, the active larvae, which are six-legged, crawl about and enter the ears of their hosts and become fully engorged in a week or two. The engorged larvae are grub-like and inactive, and after a period of quiescence, they shed their skins and turn into eight-legged nymphs, which may remain attached to their hosts for from one to seven months [Imes, 1917]. When fully engorged, the nymphs leave their host and migrate to a sheltered spot where they moult to the adult stage. In such places, both mating and oviposition occur, for, as already mentioned, the adults never feed and do not, therefore, require a host.

At the Mukteswar Laboratory, one male and one female nymph were found moulted to the adult stage on the 2nd May 1936. These were kept together in a tube and the first batch of eggs was deposited by the female about the 26th May

and the emergence of the first batch of larvae occurred on the 18th July. On the 29th July, about thirty of the larvae were introduced into the ear of a country-bred pony, the following device being adopted to prevent the ticks from escaping. The larvae were loosely held inside a small piece of muslin-gauze and the latter was then folded over to immobilize the larvae momentarily. The piece was now quickly inserted into the ear and the opening of the latter closed up by means of a piece of finely-meshed muslin glued on with Chatterton's compound. As an additional precaution, the ear, with its gauze-cover, was enclosed in a strong cloth bag, any damage to the latter being prevented by making the animal wear a specially made hood, as shown in the illustration (Plate XIV). When the ear of the animal was examined at the end of thirty-five days, four of the larvae were found to have changed to fully-engorged nymphs. A batch of larvae was also introduced into the ear of a rabbit, which was kept under observation for about a month, but the larvae died without developing. In this case, the rabbit was made to wear a card board collar to prevent it from scratching the infected ear.

Treatment

According to the Bureau of Animal Industry, United States of America, an effective remedy against ear-ticks is a mixture of two parts by volume of ordinary commercial pine tar and one part by volume of cotton-seed oil. The remedy is applied by means of a metal or hard-rubber syringe capable of holding one to two ounces, the mixture being warmed to ensure its ready flow. Story [1920] stresses the desirability of taking care that the mixture is not smeared on the hair in the ear; otherwise when the ear is flapped, the mixture may get into the eye and cause irritation.

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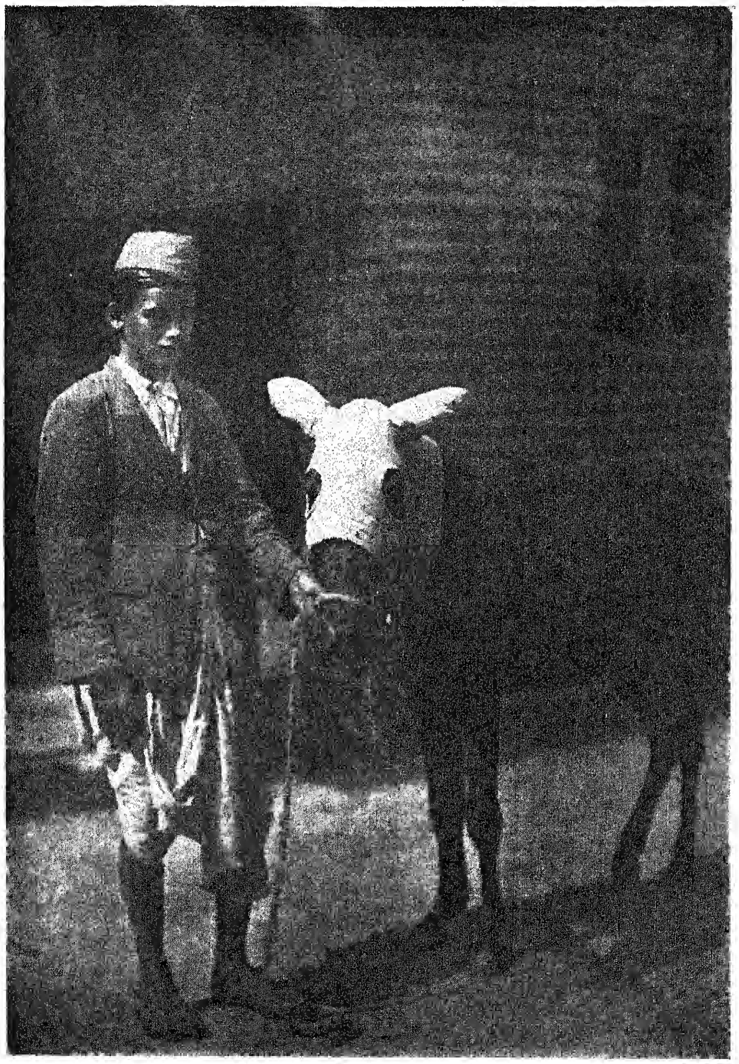

Infected pony with hood.

and the emergence of the first batch of larvae occurred on the 18th July. On the 29th July, about thirty of the larvae were introduced into the ear of a country-bred pony, the following device being adopted to prevent the ticks from escaping. The larvae were loosely held inside a small piece of muslin-gauze and the latter was then folded over to immobilize the larvae momentarily. The piece was now quickly inserted into the ear and the opening of the latter closed up by means of a piece of finely-meshed muslin glued on with Chatterton's compound. As an additional precaution, the ear, with its gauze-cover, was enclosed in a strong cloth bag, any damage to the latter being prevented by making the animal wear a specially made hood, as shown in the illustration (Plate XIV). When the ear of the animal was examined at the end of thirty-five days, four of the larvae were found to have changed to fully-engorged nymphs. A batch of larvae was also introduced into the ear of a rabbit, which was kept under observation for about a month, but the larvae died without developing. In this case, the rabbit was made to wear a card board collar to prevent it from scratching the infected ear.

Treatment

According to the Bureau of Animal Industry, United States of America, an effective remedy against ear-ticks is a mixture of two parts by volume of ordinary commercial pine tar and one part by volume of cotton-seed oil. The remedy is applied by means of a metal or hard-rubber syringe capable of holding one to two ounces, the mixture being warmed to ensure its ready flow. Story [1920] stresses the desirability of taking care that the mixture is not smeared on the hair in the ear; otherwise when the ear is flapped, the mixture may get into the eye and cause irritation.

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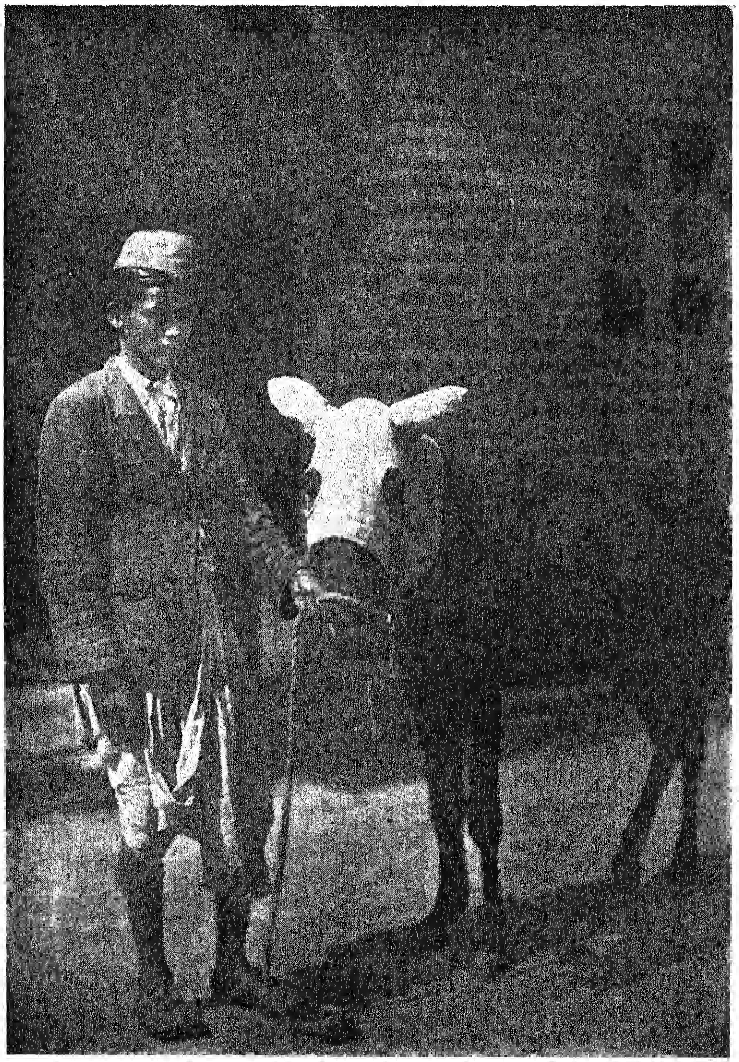

Infected pony with hood.

EXPERIMENTS ON THE TRANSMISSION OF RINDERPEST THROUGH THE AGENCY OF *STOMOXYS CALCITRANS* LINN*

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(With one text-figure)

THE possibility that biting flies are involved in the transmission of rinderpest in cattle has been stressed by more than one worker, but the work so far carried out to explore this possibility cannot be regarded as having been extensive. The results—for the most part negative—that have been obtained by different workers in transmitting the disease have already been summarized by Hornby [1926], Sen [1926] and Bhatia [1935]. A notable positive result recorded in this connection was that by Hornby, who succeeded in transmitting rinderpest in East Africa through the bites of *Glossina morsitans*. Quite recently, on a suggestion made by Crawford [1933] in Ceylon that the larger biting flies may be the transmitters of the disease in India, Bhatia [1935] carried out a series of transmission experiments with *Tabanus orientis* and *Stomoxys calcitrans*, the flies being made to bite by the so-called "interrupted" method. In the case of the former, he succeeded in obtaining positive results in one instance, but his results with *S. calcitrans* were of an entirely negative order. With the latter species, he carried out a series of four experiments, the number of flies used in each ranging from ten to fourteen. It was, however, considered desirable to carry out a more extensive series of experiments with this species before eliminating it as a transmitter of the disease. As will be seen from what has to be stated later, the results obtained by the present writers

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were negative and are, therefore, in accord with those recorded by Bhatia. Apart from reporting these results, one of the objects of this brief note is to describe a method which has proved satisfactory for the purpose of feeding a large number of flies by the "interrupted" method within a limited period of time, for it is almost needless to stress the desirability of employing a method that would substantially reduce the amount of time usually taken in carrying out feeding work of this kind and at the same time make it possible to determine, with a fair degree of certainty, what proportion of flies have actually fed.

Materials and methods

In the present trials, only wild specimens of *S. calcitrans* were used, these being of common occurrence on animals, notably buffaloes, grazing in the station area at Mukteswar during the summer months. The flies were kept singly in glass tubes measuring one inch in diameter and four inches in length, the mouths of the tubes being closed with mosquito netting with meshes (about eighteen to a linear inch) sufficiently large to permit of the flies biting through them. In order to provide moisture to the flies, the tubes containing them were kept inverted over moistened cotton wool in a tray, a sheet of wire-gauze being interposed between the tubes and the wool to prevent the flies from coming in direct contact with the moisture [*cf.* Sen, 1926]. By this means, it was possible to keep a large proportion of the flies alive for at least ten days. The mortality was high on cold, cloudy days, or when the moisture was completely evaporated off during the night and was not replenished in due time. Specimens which persistently refused to bite on any one day were allowed to suck a drop or two of cane sugar solution placed at the mouth of the tube. Each tube was numbered, so as to make it possible to maintain an accurate history of individual flies.

The flies received their infective feeds on bulls artificially infected with a virulent strain of rinderpest virus, and in all cases due care was taken to choose good reactors. After being partially fed on rinderpest-infected bulls, they were allowed to complete their feed on a young, healthy bull, housed in a small shed (*chupper*), at a convenient but safe distance from infected sheds, in order to prevent it from contracting infection by means other than through the experimental flies. Throughout the observation period, a record was kept of the morning and evening temperature of this animal, as also its general condition.

For feeding the flies, the glass tubes containing them were inserted through circular holes bored in a cork-sheet measuring 7 in. $\times$ $3\frac{1}{2}$ in. $\times$ $\frac{1}{5}$ in., the diameter of each hole being slightly less than an inch, so that the tubes fitted somewhat tightly in them. A cork-sheet of the dimensions mentioned above has been found capable of holding at least ten tubes (Fig. 1).

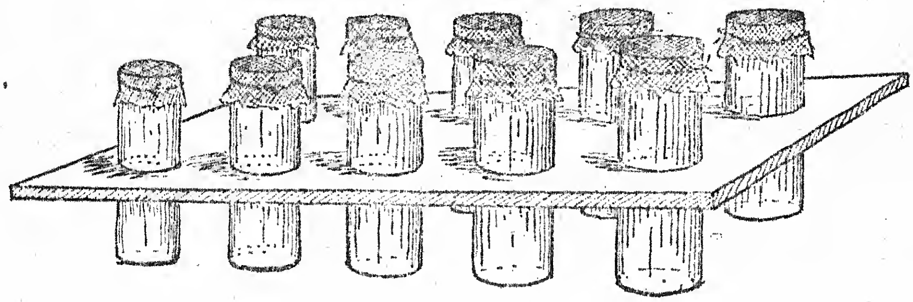

Fig. 1.—Feeding tubes arranged on cork-sheet

The animal intended to provide the infective feed was cast and immobilized by tying it down by means of a good length of rope. A fairly large area on its abdomen was now closely clipped by means of a pair of clipping scissors, and the whole set of tubes was inverted simultaneously over the clipped area, the cork-sheet being manipulated in a manner so as to cause it to assume the requisite degree of curvature, so that each fly was enabled to obtain its feed at the same time. No fly was allowed to feed for more than one minute, at the end of which the tube containing it was pulled up through the hole so as to 'break' i s contact with the body of the animal. A maximum of ten minutes was allowed to each fly to commence its feed, but it was observed that those that did not feed during the first five minutes seldom did so afterwards. It is simple for one man to operate two cork-sheets simultaneously, and by this method, the writers have, on bright sunny days, succeeded in feeding a large number of flies within a short space of time. The flies, after being infected in this manner, were allowed a maximum of ten minutes to complete their feed on a healthy animal, and seldom has any difficulty been experienced in inducing such "interrupted" feeders to feed again. The interval between the two feeds ranged from fifteen to forty-five minutes.

In the beginning, it was a matter of some difficulty to determine with certainty whether a particular fly had actually fed on the infected animal, but with experience, this became comparatively easy. The appearance of droplets of blood on the bitten spot, the 'prodding' of the proboscis through the meshes of the netting, defecation by the fly after an apparent bite—these, either singly or in combination, were taken to be diagnostic of a feed. In the case of flies that had fed partially, distension and reddening of the abdomen is not always apparent, and experience has shown that a good criterion of a feed is the occurrence of haemorrhage on the spot where the bite has been inflicted. In practice, however, it was not always easy to ascribe a haemorrhagic spot to the particular fly that had caused it. This difficulty was overcome in the following manner: A thick sheet of paper cut to the size of the cork-sheet and containing an equal number of holes of the same diameter was interposed between the cork-sheet and the clipped area. In order to ascertain whether a particular fly had fed, the cork-sheet, together with

the tubes, was partially raised without displacing the sheet of paper and the clipped area examined through the holes for the occurrence of haemorrhagic spots.

Experimental observations

As mentioned earlier in this note, only one healthy bull was used in these experiments, but, as will be seen from the tables that follow, the number of infective bites inflicted on it was sufficiently large to admit of definite conclusions being drawn as to the possibility of *S. calcitrans* being involved in the spread of the disease. In the experiments, three batches of flies were employed, those of the first two being fed singly by the method described above, but in the case of the third batch, the flies were fed *en masse*, this being considered desirable in order to hasten progress of the work, particularly when it was found that a total of as many as 411 individual bites (*infra*) failed to produce any apparent effect on the experimental animal. In the 'massive' feeding, the flies, numbering about 300, were enclosed in three cloth cages, each containing approximately 100 flies, and the cages were then applied directly on a closely clipped area on the body of an infected animal and allowed to remain there for from one to one minute and a half, and an approximate count was made of the number of flies found feeding during that period. They were then allowed to complete their feed on the healthy bull, it being assumed from previous experience gained in feeding individual flies that they had done so. The numbers of infecting bites inflicted on the healthy animal on different dates are shown in the following three tables :—

Table 1

Number of infective bites inflicted by flies of the first batch (fed singly)

	April																			May
Date	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	1
Number of bites	5	11	18	8	5	5	6	4	8	..	4	..	18	31	25	15	..	12	7	2

Total number of bites 184

Total number of days over which the bites were spread . . 17

TABLE II

Number of infective bites inflicted by flies of the second batch (fed singly)

Date	May					
	18	19	20	21	22	23
Number of bites	16	17	82	56	31	25

Total number of bites 227
Total number of days over which the bites were spread . . 6

TABLE III

Number of infective bites inflicted by flies of the third batch (fed en masse)

Date	May				June	
	28	29	30	31	1	2
Number of bites	150	120	120	80	100	100

Total number of bites 670
Total number of days over which the bites were spread . . 6

As will be seen from the foregoing tables, the experimental bull received approximately a total of 1,081 bites spread over a period of twenty-nine days, so that it may be regarded as having been offered every chance of contracting the infection through the intermediary of these flies. The animal, however, remained normal during an observation period of about forty-five days, commencing from the date on which it received its last bite. At the end of this period, it was inoculated with 5 c.c. of virulent rinderpest blood, and as a result it died after developing typical symptoms of the disease.

Summary and conclusions

1. *Stomoxys calcitrans* has been found incapable of transmitting rinderpest under experimental conditions.

2. A method is described for feeding a large number of flies singly, by the "interrupted" method, within a short period of time.

Acknowledgments

We are deeply grateful to Mr. F. Ware, C. I. E., F. R. C. V. S., F. N. I., I. V. S., Director of the Imperial Veterinary Research Institute, Mukteswar, for granting facilities in the prosecution of the investigation. The junior author is indebted to the Civil Veterinary Department, Punjab, for the grant of a research scholarship, during the tenure of which this work was carried out.

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SOME DIGESTIBILITY TRIALS ON INDIAN FEEDING STUFFS

XI. COTTON-SEED CAKE AS A CATTLE FEED

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In earlier publications [Lander and Dharmani, 1929, 1935] of this series, an account was given of the feeding values of certain American and *Desi* cotton-seeds as determined by digestibility trials at Lyallpur using oat hay as a basal ration. One of the objects of those investigations was to ascertain how far the prevailing prejudice against American cotton-seed as a feeding material on account of the adhering lint held good. It was shown that this prejudice could not be substantiated. It was also suggested [Lander and Dharmani, 1929] that an oil-content as high as that found in cotton-seed, *viz.*, about 20 per cent was unnecessary for cattle, and that perhaps a greater utilization of the feed could be made if cotton-seed cake instead of cotton-seed were fed. No trials at that time could, however, be conducted with cake as it was not then possible to obtain cotton-seed cake in the Punjab. With the expansion of the soap industry in the Province a great demand for cotton-seed oil has arisen and a local mill has recently produced cotton-seed cake from 4 F American cotton seeds which are comparatively less fuzzy than other American varieties. The cake is produced by pressing the whole seed, without delinting or decorticating, in the expellers, from which the cake issues in fine flakes which resemble *toria* (*Brassica napus*) cake in appearance. This cotton-seed cake has been used to a large extent by the Military Dairy Department, but has not yet been taken up by farmers although it is selling in Lyallpur at the cheap rate of from ten to fourteen annas a maund.

The value of cotton-seed cake as a cattle feed is well recognised in Europe and America, but no definite data based on controlled feeding trials regarding the feeding value of cotton-seed cake manufactured in the province are available except some preliminary observations made by S. Kartar Singh in 1915.

In order to investigate the feeding value of this new concentrate, digestibility trials were carried out at Lyallpur on three Montgomery breed heifers with oat hay (a maintenance ration) as basal ration fed *ad lib.* The technique followed during the experiments was the same as already published [Lander and Dharmani, 1929].

Cotton-seed cake was fed once a day separately after being soaked in water for about two hours prior to feeding. Two heifers were given 2·5 lb. of cotton-seed cake each per day while the third was fed 2·0 lb. only. The slight variations in the quantity of cottons-eed cake fed were made in order to see whether such variations would have any effect on the digestibility figures obtained. There was no appreciable difference to record. No untoward effects whatsoever were noticed in the general health of the experimental animals over long periods.

Table I shows the chemical composition of the cotton-seed cake, and for comparison, corresponding data for *Sarson* (*Brassica campestris*) and *toria* cakes, which are commonly used in the Punjab, are also shown.

It will be seen that cotton-seed cake contains a lower percentage of fat and protein than the other cakes and a larger amount of crude fibre.

The digestibility data for various cakes given in Table I show that the digestibility coefficients for dry matter, fat and fibre are higher in cotton-seed cake than in the others.

A perusal of the data set forth in Table II showing starch equivalents, digestible protein, albuminoid ratio and price per unit, reveals that cotton-seed cake is slightly lower in starch equivalents than other cakes, and is markedly lower in digestible protein. It is, however, the cheapest of all the cakes from the point of view of starch equivalents and digestible protein and it would pay to buy and feed cotton-seed cake, assuming that the proteins in various cakes are of equal biological efficiency. Even if the price of the cotton-seed cake rises to Rs. 1-10-6 per maund it would still be an economical feed to employ.

Table I

—	Chemical composition per cent on feed as such 1							Chemical composition per cent on oven dried feed 2				
	Moisture	Dry matter	Ash	Fat	Fibre	Protein	Nitrogen free extract	Ash	Fat	Fibre	Protein	Nitrogen free extract
Cotton-seed cake	7·48	92·52	6·01	8·47	22·31	21·13	34·60	6·50	9·15	24·11	22·84	37·40
Sarson cake	8·74	91·26	9·12	10·10	9·17	32·94	29·93	9·99	11·11	10·05	36·09	32·76
Toria cake	5·31	94·69	7·14	11·83	10·61	32·00	33·11	7·54	12·49	11·20	33·79	34·98

Table I—*contd.*

—	Digestibility coefficients 3						Digestible nutrients per 100 lb. of the feed 4					
	Dry matter	Ash	Fat	Fibre	Protein	Nitrogen free extract	Dry matter	Ash	Fat	Fibre	Protein	Nitrogen free extract
Cotton-seed cake	72·81	68·78	98·01	73·66	85·03	59·36	67·36	4·13	8·30	16·43	17·97	20·53
Sarson cake	70·12	...	93·08	43·62	85·00	73·50	64·00	...	9·40	4·00	28·00	22·00
Toria cake	70·39	...	90·84	38·09	84·37	60·74	66·65	...	10·75	4·04	27·00	20·11

TABLE II

Name of the feed	Starch equivalents	Digestible protein per 100 lbs. of the feed	Albuminoid ratio 1 :	Price per starch equivalent based on current prices at Lyallpur in pies	Price per pound of digestible protein based on current prices at Lyallpur in pies
Cotton-seed cake	60·56	17·97	3·1	2·0	6·7
Sarson cake	69·35	28·00	1·7	7·1	17·6
Toria cake	69·52	27·00	1·8	7·1	18·2

Table III

—	Chemical composition per cent on feed as such 1							Chemical composition per cent on oven dried feed 2				
	Moisture	Dry matter	Ash	Fat	Fibre	Protein	Nitrogen free extract	Ash	Fat	Fibre	Protein	Nitrogen free extract
Cotton-seed cake	7·48	92·52	6·01	8·47	22·31	21·13	34·60	6·50	9·15	24·11	22·84	37·40
4 F. American cotton-seed . . .	6·71	93·29	4·63	20·73	20·96	17·50	29·47	4·96	22·23	22·47	18·76	31·58

Table III—*contd.*

—	Digestibility coefficients 3						Digestible nutrients per 100 lbs .of the feed 4						(On feed as such) 5		
	Dry matter	Ash	Fat	Fibre	Protein	Nitrogen free extract	Dry matter	Ash	Fat	Fibre	Protein	Nitrogen free extract	Starch equivalent	Protein per 100 lbs.	Albuminoid ratio 1 :
Cotton-seed cake	72·81	68·78	98·01	73·66	85·03	59·36	67·36	4·13	8·30	16·43	17·97	20·53	60·56	17·97	3·1
4 F. American cotton-seed	52·04	...	87·90	38·10	60·37	30·11	48·54	...	18·23	7·99	10·57	9·03	62·85	10·57	5·5

Table III—*concld.*

—	Digestible nutrients per 100 lb. of oven dried feed 6						(On oven dried feed) 7		
	Dry matter	Ash	Fat	Fibre	Protein	Nitrogen free extract	Starch equivalents	Protein per 100 lbs.	Albuminoid ratio 1 :
Cotton-seed cake . . .	72·80	4·46	8·97	17·76	19·42	22·19	65·46	19·4	3·1
4 F. American cotton-seed . .	52·03	...	19·54	8·57	11·33	9·68	67·37	11·4	5·5

In Table III, the values of cotton-seed have been compared with those of cotton-seed cake. The figures for digestible coefficients, starch equivalents and digestible protein show that the nutrients in the form of cake have been better utilized by the animals than in the form of seed.

TABLE IV

Name of the feed	Fat percentage	Digestibility coefficients of fat	Digestibility coefficients of fibre
Sarson seed (crushed)	35·22	82·33	Negative
4 F. cotton-seed	20·73	87·90	38·10
Toria cake	11·83	90·84	38·09
Sarson cake	10·10	93·08	43·62
Cotton-seed cake	8·47	98·01	73·66

Table IV shows data which indicate that the digestibility of fat and fibre in oil seeds and cakes is related in some degree to their fat content. While the digestibility of fat increases rather steadily with the fall of the fat-content, the digestibility of the fibre tends to increase irregularly. A fat-content about 8 per cent seems to be the most desirable one from the point of view of maximum utility of fat in oil-seeds and cakes.

Acknowledgment is made of the assistance given in this work by N. Akbar Ali of the Chemical Section.

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A NOTE ON A VARIETY OF *SALMONELLA ENTERITIDIS* ISOLATED FROM PIGEONS

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(With Plate XV and two charts)

Introduction

Outbreaks of septicaemia in pigeons due to *S. enteritidis* Gaertner are of rare occurrence judging by the few recorded cases in literature and the recording of an outbreak of this disease in pigeons maintained for vaccine production at this Institute is, therefore, of special interest. It may be mentioned that no previous records exist, in India, to establish the incidence of salmonellosis in pigeons and it is believed that this, if not the first outbreak, is, at least, the first in which a detailed investigation has led to definite conclusions regarding the etiology of pigeon septicaemia.

The pigeons which were the victims of the attack were normally used for the production of fowl-pox vaccine. It was noted that, shortly after the virus inoculations, pigeons rapidly commenced to die, the symptoms noted being fever and enteritis of an acute type. As the symptoms could not be ascribed to the virus inoculations, a bacteriological examination of the diseased birds was made. A strain of *S. enteritidis* was readily isolated from the blood and organs of every pigeon examined, and on further biochemical and serological tests was proved to be *S. enteritidis* Gaertner. Significance attaches to the finding since pigeons are a popular and easily obtainable article of diet in India and as the organism in question is pathogenic for man, the potential danger of diseased or 'carrier' birds is readily realised.

Materials and methods

The organism described was isolated in all cases from the heart-blood of pigeons after death, and four cultures were used for the experimental work. The numbers 124, 125, 127 and 115 refer to the numbers of the pigeons from which they were obtained. The cultures were subjected to a primary pathogenicity test and purified after repeated plating. No difficulty was noted in obtaining

pure cultures to proceed with. The various anti-sera used for typing the Salmonella, under study, were prepared from rabbits and the antigens from authentic stock strains.

TABLE I

Details of the cultures and anti-sera used for studying the agglutination reactions

No. of culture	Particulars regarding source	Anti-serum prepared from	Remarks
124	Pigeon inoculated with pox virus on 15th Oct., 1936 and died on 30th Oct., 1936.	None	No lesions of pox.
125	Pigeon inoculated with pox virus on 15th Oct., 1936 and died on 25th Oct., 1936.	Do.	Do.
127	Pigeon inoculated with pox virus on 15th Oct., 1936 and died on 31st Oct., 1936.	Rabbit	Pox lesions reproduced.
115	Pigeon inoculated with live culture isolated from Pigeon No. 124 (above) on 31st Oct., 1936 and died on 8th Nov., 1936.	Do.	More pigeons inoculated along with this, died and Salmonella recovered (Table IV).
S. 52	Lister Institute . . .	Do.	Fowl typhoid (*s. gallinarum*).
S. 53	Do. . . .	Do.	*S. pullorum.*
S. 44	Do. . . .	Do.	*S. enteritidis* Gaertner.
S. 51	Do. . . .	None	*Paracoli* (*enteritidis*).
S. 63	Local	Do.	*S. enteritidis* from the Pathology Department of this Institute.
S. 65	Local Spec. No. 537/32 Abortion material brood mare.	Do.	*S. enteritidis.*
S. 84	Lister Institute . . .	Do.	*S. enteritidis* Limerick.
S. 85	Do. . . .	Do.	*S. enteritidis* var. Danzig.
S. 86	Do. . . .	Do.	*S. enteritidis* var. Dublin.
S. 87	Do. . . .	Do.	*S. enteritidis* var. Rostock (Kauffmann).
S. 88	Do. . . .	Do.	*S. enteritidis* var. Moscow.
S. 97	Specific *enteritidis* in calves. Spec. No. 564/34.	Rabbit	*S. enteritidis* var. Dublin (Indian strain).

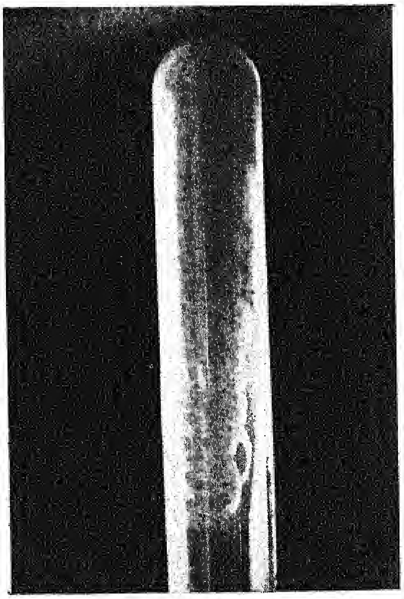

Fig. 1 Twenty-four hour old agar culture of *Salmonella* from pigeons.

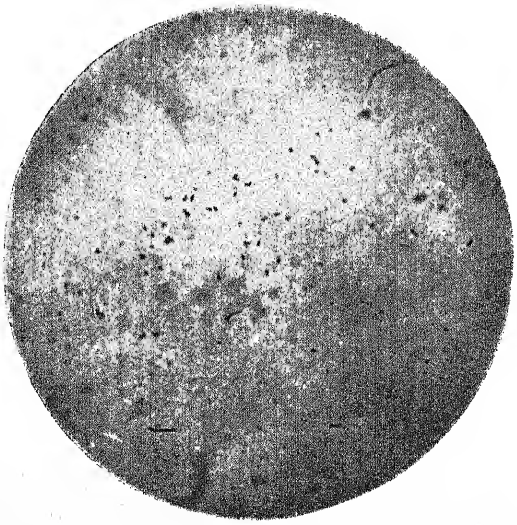

Fig. 2 Smear stained by Gram's method, 24-hour old agar culture.

Morphology and staining characteristics

The organisms are indistinguishable from members of the Colon-typhoid group. The rods measure from 1·5 to 2·0 microns in length and fro n 0·5 to 0·8 of a micron in breadth. From agar cultures singles and pairs are seen. (Plate XV. Fig. 2.) Under dark ground illumination many pairs could be demonstrated with pronounced motility. It is gram-negative.

Cultural characteristics

Growth is luxuriant under aerobic conditions in media with a *p*H of 7·4. It propagates well at 37° C. in the incubator but at room temperature (14° C.) growth is rather poor.

Agar.—Colonies on agar plate are circular with regular outline, moist and of a greyish colour. The growth on agar slant is fairly heavy after eighteen hours at 37° C., moist and greyish and easily removable by platinum loop. (Plate XV, Fig. 1.)

Gelatin.—In stab cultures the growth occurs on the surface and along the stab. There is no liquefaction.

Broth.—Broth is rendered uniformly turbid. In twenty-four hours culture grown at 37° C., sedimentation and light grey pellicle formation are seen.

Litmus milk.—Acid is produced during the first twenty-four hours. No clotting occurs.

Peptone solution.—Indol is not produced. Twenty-four to seventy-two hours cultures grown at 37° C. were tested for the presence of indol by the addition of Ehrlich's reagent.

Nitrate solution.—This is reduced after eighteen hours growth at 37° C.

Acetyl-methyl-carbinol.—Not formed.

H_2S.—The culture is subcultivated on liver infusion broth agar with a strip of filter-paper, impregnated with lead acetate 10 per cent solution, at the mouth of the tube. The production of H_2S is marked after twenty-four hours incubation at 37° C., as evidenced by the blackening of the lead acetate paper.

Resistance

As has been reported by various workers, members of the Salmonella group are not very resistant to heat. Four 24-hours old cultures from different pigeons were emulsified in normal saline solution, and the live bacterial emulsions thus prepared were subjected to heat in a water-bath, maintained at 58° C. for thirty minutes. At intervals of ten minutes, the heated material was sown on plain agar slants and incubated for seventy-two hours. In all cases the cultures were dead after thirty minutes' heating at 58° C.

Table II

Results of heating live-cultures at 58° C. in water-bath

Culture from pigeon No.	Results of sowing heated cultures on agar slants		
	10 minutes	20 minutes	30 minutes
115	Alive . . .	Sterile . . .	Sterile.
124	Sterile . . .	Do. . .	Do.
125	Do. . .	Do. . .	Do.
127	Alive . . .	Alive . . .	Do.

Fermentable media

As sugar fermentation tests afford a fairly accurate means of differentiating the various species of organisms under the Salmonella genus, a number of carbohydrates were tested. Andrades' indicator was incorporated in the media for the detection of acidity. The tubes were incubated at 37° C. for ninety-six hours and the final readings then recorded. In most cases acid and gas formation were demonstrated after twenty-four hours except in xylose where fermentation occurred only after forty-eight hours. The four cultures of pigeon Salmonella as isolated from the heart-blood after death are detailed in Table III with the fermentation reactions. The usual symbols of "A" for acid and "A. G," for acid and gas are used.

TABLE III

Showing fermentation reactions of Salmonella from pigeons

Name of medium	Cultures from pigeon numbers			
	124	125	127	115
Dextrose	A. G.	A. G.	A. G.	A. G.
Lactose	Not attacked	Not attacked	Not attacked	Not attacked.
Sucrose	Not attacked	Not attacked	Not attacked	Not attacked.
Mannite	A. G.	A. G.	A. G.	A. G.
Xylose	A. G.	A. G.	A. G.	A. G.
Inosite	Nat attacked	Not attacked	Not attacked	Not attacked.
Dextrin	A. G.	A. G.	A. G.	A. G.
Raffinose	Not attacked	Not attacked	Not attacked	Not attacked.
Maltose	A. G.	A. G.	A. G.	A. G.
Levulose	A. G.	A. G.	A. G.	A. G.
Dulcite	A. G.	A. G.	A. G.	A. G.
Arabinose	A. G.	A. G.	A. G.	A. G.
Rhamnose	A. G.	A. G.	A. G.	A. G.
Sorbite	A. G.	A. G.	A. G.	A. G.
Salicin	Not attacked	Not attacked	Not attacked	Not attacked.

According to Bergey and other standard books on "Determinative Bacteriology", the cultures from the four different pigeons are identical, and may be classified as *S. enteritidis*.

As the creation of a new species under a particular genus would add confusion to the existing nomenclature, care was taken to type the species, under study, with already known members after a careful study of the cultural and biochemical characteristics of the organism. It seems reasonable to assume the pigeon strain to be one of *S. enteritidis* Gaertner.

PATHOGENICITY

As stated in Table I, pigeon No. 115 was injected with the organism isolated from pigeon No. 124 and after death, the same organism was recovered. Pigeons were inoculated subcutaneously with the culture as well as with organ emulsion

from dead pigeons, with fatal results, in many cases attended with a rise in temperature and persisting diarrhoea. (Table IV). A dose of 1 c.c. of a saline emulsion of agar surface growth twenty-four hours old, containing about 300 millions bacteria per c.c. was injected while testing the pathogenicity and death occurred invariably after a week. A rabbit, when intravenously injected, died after forty-eight hours, and marked fever and diarrhoea were seen.

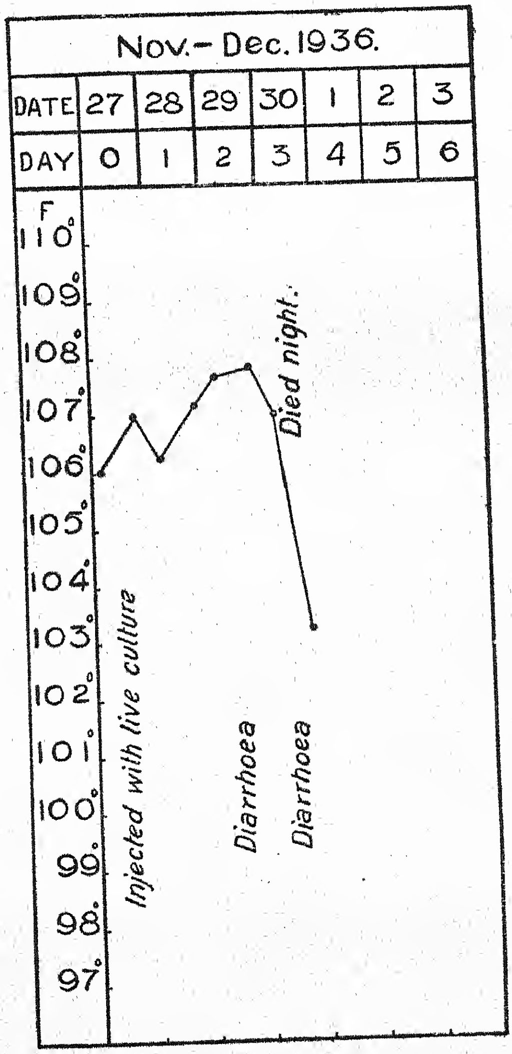

CHART No 1.

Temperature chart of Pigeon No. 146 injected with live-culture of *Salmonella* isolated from Pigeon No. 115.

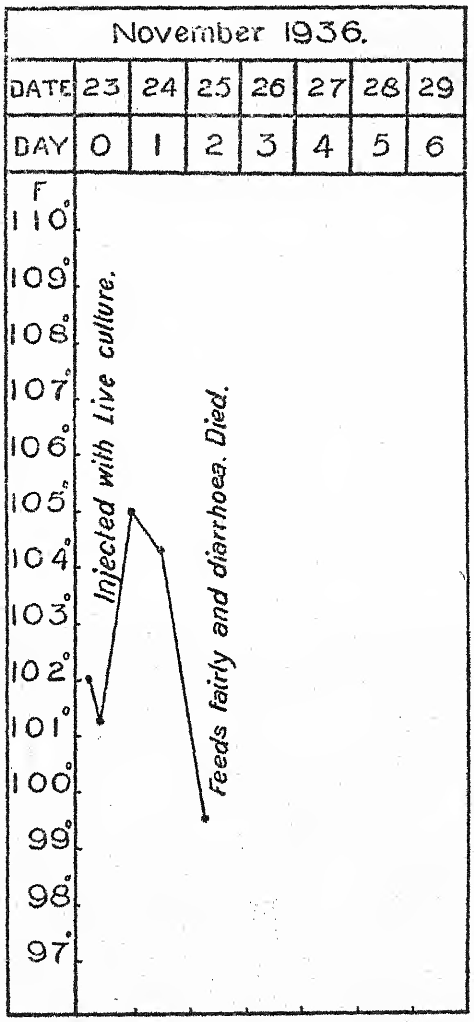

CHART No. 2.

Temperature chart of Rabbit No. 103 injected intravenously with the *Salmonella* from Pigeon No. 115.

TABLE IV

No. of pigeon inoculated	Date of inoculation	Material inoculated	Route of injection	Result	Remarks
113	30th October, 1936	Organ emulsion from pigeon No. 124.	Subcut.	Died on 13th November, 1936.	Salmonella recovered.
114	Do.	Do.	Do.	Survived.	—
115	31st October, 1936	Culture from pigeon No. 124 (Salmonella).	Do.	Died on 8th November, 1936.	Salmonella recovered.
116	Do.	Do.	Do.	Died on 13th November, 1936.	Do.
145	27th November, 1936	Culture from pigeon	Do.	Died on 3rd December, 1936.	Do.
146	Do.	Do.	Do.	Died on 1st December, 1936.	Do.
147	Do.	Culture from pigeon No. 124 (Salmonella).	Do.	Died on 2nd December, 1936.	Do.
148	Do.	Do.	Do.	Died on 5th December, 1936.	Do.
149	Do.	Culture from pigeon No. 125 (Salmonella).	Do.	Died on 4th December, 1936.	Do.
150	Do.	Do.	Do.	Died on 15th December, 1936.	Do.
151	Do.	Culture from pigeon No. 127.	Do.	Survived.	—
152	Do.	Do.	Do.	Died on 5th December, 1936.	Salmonella recovered.

Rabbits were immunised for the production of anti-sera. Immune sera for two strains of Salmonella, *S. pullorum*, *S. gallinarum*, *S. enteritidis* Gaertner (S. 44) and *S. enteritidis* var. Dublin (S. 97) from specific enteritis in calves (Table I), were available for studying the serological reactions by agglutination tests.

While conducting agglutination tests, care was taken to eliminate spontaneous clumping of the antigen by the proper standardisation of the antigen and the employment of antigen *plus* saline control tubes. In all tests there was no difficulty in securing a stable antigen. The results were read after incubation at 37°C. for twenty-four hours. At first the homologous titres of the anti-sera were determined. Later, cross tests were made to study the immunological identity. When interpreting the results of agglutination reaction the degree of antigen antibody union was noted and recorded by the use of symbols, *C*, *P*, and *T* which

represent complete, partial and trace agglutination respectively. The homologous titres of the samples of immune sera, when submitted to test, are given in Table V.

TABLE V

Homologous agglutinin titres of immune sera

Particulars of anti-serum	Antigen	Agglutinin titre after incubation for 24 hours at 37°C. Dilutions 1/10.	1/50.	1/100.	1/500.	1/1,000.	1/5,000.	1/10,000.	Antigen control	Remarks
Pigeon No. 115 Salmonella .	Homologous .	C	C	C	C	C	C	—	—	
Do. do. .	Pigeon No. 127	C	C	C	C	C	P	—	—	
Pigeon No. 127 Salmonella .	Homologous .	C	C	C	C	C	P	—	—	
Do. do. .	Pigeon No. 115	C	C	C	C	P	T	—	—	
S. 44. *Salmonella enteritidis* Gaertner.	Homologous .	P	—	—	C	C	C	C	—	Prezone reaction.
S. 97. *S. enteritidis* var. Dublin. Calf enteritis.	Do. .	P	C	C	C	C	C	P	—	Do.
S. 53. *S. pullorum* . .	Do. .	C	C	C	C	C	P	—	—	
S. 52. *S. gallinarum* .	Do. .	C	C	C	C	C	T	—	—	

CROSS AGGLUTINATION TESTS

Pigeon Salmonella Antigen vs. *authentic strains of Salmonella anti-sera*

Agglutination tests conducted on four different pigeon Salmonella antigens, whose fermentation reactions were similar, with two samples of anti-sera for *S. enteritidis* (S. 44 and S. 97), *S. gallinarum* and *S. pullorum* are shown in Table VI. It indicates that there is some relationship with S. 97 (calf enteritis) and that the poor agglutinin titres with S. 44, *S. pullorum* and *S. gallinarum* immune sera, are due to the presence, in the sera, of group agglutinins.

TABLE VI

Pigeon Salmonella antigens vs. *anti-sera for different Salmonella species*

Particulars of anti-serum	Antigen Salmonella from	Agglutination titre after incubation for 24 hours at 37°C. Dilutions of serum 1/10.	1/50	1/100.	1/500.	1/1,000.	1/5,000.	1/10,000.	Antigen control
S. 97. *S. enteritidis* var. Dublin Calf.	Pigeon No. 115	C	C	C	P	—	—	—	—
Do. do.	„ No. 124	C	C	C	T	—	—	—	—
Do. do.	„ No. 125	C	C	C	T	—	—	—	—
Do. do.	„ No. 127	C	C	C	T	—	—	—	—
S. 44. *S. enteritidis* Gaertner	„ No. 115	C	P	—	—	—	—	—	—
Do. do.	„ No. 124	C	P	—	—	—	—	—	—
Do. do.	„ No. 125	C	—	—	—	—	—	—	—
Do. do.	„ No. 127	C	—	—	—	—	—	—	—
S. pullorum from Rabbit No. 162	„ No. 115	C	P	—	—	—	—	—	—
Do. do.	„ No. 124	C	P	—	—	—	—	—	—
Do. do.	„ No. 125	P	T	—	—	—	—	—	—
Do. do.	„ No. 127	P	P	—	—	—	—	—	—
S 52. *S. gallinarum* from Rabbit No. 160.	„ No. 115	C	P	—	—	—	—	—	—

From the above table, it is seen that pigeon Salmonella bears no relationship to *S. pullorum* and *S. gallinarum*, the two pathogenic species incriminated in fowl epidemics, a possibility which has been ruled out by the biochemical tests.

AGGLUTININ ABSORPTION TEST

Two samples of anti-sera for S. 44 and S. 97 (Table I) were first absorbed by the Salmonella antigen from pigeon No. 115 independently, by the following method. Into two centrifuge tubes of 10 c.c. capacity, 2·5 c.c. of the dilution of the respective serum was made and then 2·5 c.c. of a thick saline suspension of twenty-four hours old agar surface growth of pigeon No. 115 Salmonella added. The antigen-antibody mixture was then kept at 55°C. in an electrically heated water-bath for four hours after which the tubes were centrifuged at 2,000 R. P. M. for twenty minutes to sediment the bacterial mass. The supernatant fluid was separated and further agglutination tests were conducted with the absorbed sera against their homologous antigens and the pigeon Salmonella for evidence of absorption on the original homologous titres.

As recorded in Table VII, S. 97 anti-serum when absorbed by pigeon No. 115 Salmonella, lost a considerable amount of its agglutinating anti-body for the specific antigen and this suggests some similarity between the agglutinogen fraction of the two. The titre was unaffected in the case of absorption of S. 44.

TABLE VII

Agglutinin absorption tests

Particulars of serum	Antigen used for absorption	Agglutinin titre after incubation for 24 hours at 37° C.	Remarks
Anti-serum for *S. enteritidis* Gaertner. S. 44, absorbed by pigeon No. 115, Salmonella organism.	Pigeon No. 115 Salmonella.	Neg. 1/20 .	—
Do. do. .	Specific . .	1/5,000 .	Original homologous titre with unabsorbed serum 1/10,000.
Anti-serum for *S. enteritidis* var. Dublin (calf enteritis) S. 97, absorbed by pigeon No. 115, Salmonella organism.	Pigeon No. 115 Salmonella.	Neg. 1/20 .	—
Do. do. .	Specific . .	1/1,280 .	Original homologous titre with unabsorbed serum 1/10,000.

CROSS AGGLUTINATION TEST

Pigeon Salmonella anti-serum vs. *various authentic types of Salmonella antigens*

Having determined the agglutinin titre of pigeon No. 115 organism anti-serum against the specific antigen, *viz.*, 1/5,000 the same serum was tested against a number of authentic strains of the Salmonella group, stocked at this Institute for comparative study and from the titres so obtained, Table VIII indicates that S. 63 and S. 65 cultures are highly suggestive of the serological relationship with the Salmonella from the pigeons referred to in this paper. S. 63 and S. 65 were locally isolated strains of *S. enteritidis* from animal sources.

TABLE VIII

Cross agglutinin titre of pigeon No. 115. Salmonella anti-serum against various types of authentic Salmonella antigens

No.	Antigen Type of organism	Agglutinin titre after incubation for 24 hours at 37°C. Dilutions of the serum							Antigen control
		1/10	1/50	1/100	1/500	1/1,000	1/5,000	1/10,000	
S. 44	*S. enteritidis* Gaertner	T	...	...	...	...	...	...	...
S. 97	*S. enteritidis* var. Dublin	P	...	...	...	...	...	...	...
S. 51	*Paracoli enteritidis*	C	P	...	...	...	...	...	...
S. 63	*S. enteritidis* from the Pathology Department of this Institute.	C	C	C	C	C	P	...	...
S. 65	*S. enteritidis*, abortion material from brood mare.	C	C	C	C	C	C	P	...
S. 85	*S. enteritidis* var. Danzig	C	T	...	...	...	...	...	...
S. 86	*S. enteritidis* var. Dublin	P	T	...	...	...	...	...	...
S. 87	*S. enteritidis* var. Rostock (Kauffmann).	T	...	...	...	...	...	...	...
S. 88	*S. enteritidis* var. Moscow	C	T	...	...	...	...	...	...
S. 84	*S. enteritidis* Limerick	C	C	C	P	...	...	...	...
S. 93	*S. anatum*	C	P	T	...	...	...	...	...
S. 52	*S. gallinarum*	...	...	...	...	...	...	...	...
S. 53	*S. pullorum*	P	...	...	...	...	...	...	...

SUMMARY AND CONCLUSIONS

1. In a batch of pigeons inoculated with experimental pigeon-pox virus at this Institute, deaths from a septicæmic disease occurred and from the heart-blood of such pigeons, a gram negative bacterium was isolated.

2. The cultures from the diseased pigeons were, on their cultural and pathogenic merits, subjected to a detailed study.

3. Agglutination, cross agglutination and agglutinin absorption tests conducted with authentic types of the genus Salmonella suggest a marked serological relationship with two stock strains of *S. enteritidis* isolated from animals at this Institute.

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ON A PIROPLASM OF THE INDIAN CAT (*FELIS DOMESTICUS*)*

BY

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(With Plate XVI)

ON the 17th March 1936, the carcase of one of a number of cats obtained from Bareilly (U. P.) for experimental work at the Imperial Veterinary Research Institute, was received for autopsy in the post-mortem room. The animal was in a poor condition; otherwise it showed no external signs of disease. Apart from a general somewhat anaemic condition, the internal organs showed little of interest superficially. Slight congestion of the kidneys, however, was noticed. The bowels, too, showed hyperaemic patches. No evidence of icterus or haemoglobinuria was seen at autopsy. From the helminthological point of view, the carcase was full of interest as it harboured the following different parasites in varying numbers :—

Lungs—*Paragonimus westermanni*.
Liver—*Opisthorchis felineus*.
Stomach—*Chlamydonema praeputiale*.
Bowels—*Echinochasmus perfoliatus*.
Mesocestoides lineatus.
Rictularia cahirensis.
Taenia taeniaeformis.
Toxocara mystax.

No ectoparasites were found.

In view of the rich parasitic fauna, the author expected that examination of blood would reveal some protozoan parasites or micro-filariae, and hence several smears from the peripheral blood were prepared and a sample of heart-blood collected. The smears were stained by Leishman's method and microscopically examined. A careful search for micro-filariae proved negative, but the effort was rewarded by the detection of non-pigmented protozoan parasites in the red blood corpuscles. Judging from the small number of erythrocytes which showed the

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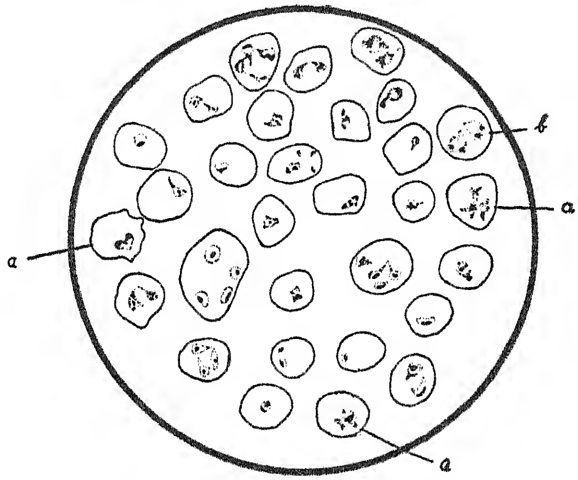
parasites, the infection was by no means of a severe degree ; so that many fields had to be examined before it was conclusively ascertained that the parasite was a piroplasm. The accompanying diagram (Plate XVI) drawn with the aid of the camera lucida shows a representative group of infected red blood corpuscles collected from different fields.

The individual parasite was small, being on an average 1·5 μ the actual size varying from less than one μ to 2·50 μ. Morphologically it resembled to some extent the one described by Davis [1929] as *Babesia felis* from the Sudanese wild cat (*Felis ocreata*). Small and large, rounded or ovoid forms, sometimes vacuolated (ring-forms), were common, one or more (up to four) being present in one red blood corpuscle. Amoeboid and dividing forms (Plate XVI, *a*) were very rare. Not infrequently typical pear-shaped parasites were seen. These were either single or, as was more often the case, in pairs ; sometimes the pair was markedly divergent as in *Babesia bovis* ; rarely three or four parasites were seen in one red blood corpuscle. In no instance, however, a cruciform arrangement suggestive of division into four, as seen by Davis and by Wenyon and Hamerton [1930] was noticed. The chromatin had no fixed place in the parasite but was inclined to be peripherally situated in the rounded forms and towards the blunt end in the pear-forms. Anaemic changes were observed in the blood smears.

Realising that the material was not fresh, blood smears were made from time to time from the other living and apparently healthy cats, and a careful search was made for piroplasms in their blood smears and for ectoparasites on their bodies. The result was negative in every case. But the presence of intracorpuscular bodies resembling *Anaplasma marginale* was detected in the blood smears of all the cats. The finding of these bodies is in conformity with that of Davis [1929] who doubts if they " bear any relation to the piroplasm " and adds " whether they are actual parasites or merely Jolly bodies it is difficult to determine ".

Lingard and Jennings in 1904 mentioned the occurrence of piroplasms in various species of animals including lizards, fowls, cats, camels and elephants. They even went to the extent of recording this parasite from a man. These observations have not been confirmed and for certain reasons their article is open to much criticism. From their coloured plates and description of some of the parasites it seems highly probable that the authors were, in some cases, dealing with what were really not piroplasms ; indeed, in the human case the parasites appear to be *Plasmodium vivax*. Unfortunately their " preliminary note " was never modified, as they had proposed to do. No description or illustration of their feline piroplasm is available and there appears, therefore, enough justification to believe with Davis [1929] that until the latter's paper appeared in 1929 no piroplasm had " been found in any member of the Felidae ".



Representative group of infected red blood corpuscles collected from different fields.

In 1930, Wenyon and Hamerton described from the Bay Lynx (*Felis rufa*) a piroplasm which "resembles closely" *Babesia felis* of Davis. Subsequently Carpano [1934] recorded a new piroplasm from the Puma (*Felis concor*) resembling *B. gibsoni*, and asserted that it too was transmitted from the local felines (presumably including *F. domesticus* and *F. ocreata*), which were carriers, by the tick *Rhipicephalus simus*. He proposed the name *Babesiella felis* for his parasite and would have the parasite of Davis called *Nuttallia felis*, perhaps because it produces cross-forms. But Wenyon points out: "the difference in size and in the number of daughter individuals produced are not sufficient grounds for the recognition of a new genus". It is evident, however, that Carpano was dealing with a parasite entirely different from the one described by the author and by Wenyon and Hamerton.

There appears to have been no further publication on feline piroplasms. In the present case, as already pointed out, a material difference from *B. felis* of Davis is shown by the absence of cross-forms, division being into two daughter parasites. The occurrence of cross-forms is corroborated by Wenyon and Hamerton in their description of the parasite from *F. rufa*. They leave little doubt as to the mode of division of the parasite into four daughter individuals, which is similar to that in *B. equi*. They admit, however, that even in their heavily infected case "the cross-form are few in number, but smaller forms showing division into two daughter individuals were fairly frequently seen". From this it appears that in the Bay lynx the parasite divides usually into two, the cross-shaped arrangement resulting from a departure from this normal mode of division.

Since in the Indian cat no evidence of division into four, or the presence of cross-forms, was encountered, the rare occurrence of four parasites in one erythrocyte may have arisen much as it sometimes does in *Babesia bigeminum* or *B. canis*. Indeed, the author has seen, although not commonly, infected red blood cells (Plate XVI, b) showing coupled pear-shaped parasites one or both of which are suggestive of stages preparatory to further division.

From the description and illustrations of *B. felis* of Davis and from the suggested resemblance of the piroplasm of Carpano with *B. gibsoni*, it would appear that the two parasites are similar but entirely different from those described from *F. rufa* by Wenyon and Hamerton, and from the Indian cat in this paper. It is, however, not clear why, in spite of the evidence of division, "fairly frequently", of the parasite from *F. rufa* into double forms, Wenyon and Hamerton have found it comparable to *B. felis* [Davis, 1929] wherein no such forms have been described or illustrated. The division into four as described by Wenyon and Hamerton was entirely wanting in the Indian cat, and therefore, the author believes his parasite to be different from those hitherto described but refrains, in the absence of suitable material to carry out further studies by inoculation work, from referring it to a new species.

ACKNOWLEDGMENT

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DETERMINATION OF FATS IN BIOLOGICAL MATERIAL

II.—THE ALKALI HYDROLYSIS METHOD*

BY

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THE alkali-hydrolysis method originally developed by Liebermann [Liebermann and Szekeley, 1898] finds very common adoption in the technique of fat-determination in biological material. Other modifications of this method such as that of Kumagao and Suto [1908], have for their object only a thorough breaking down of the tissues to enable the ether or other extracting solvent to have a ready access to the fat molecules of the substance. It should also be added that this method is considered particularly useful when it is specially desired to avoid the changes or losses in fat that generally occur on drying the material.

During recent years there seems to be a comparative paucity of reported work on the testing of fat-determination methods. In the first paper of this series [Seshan, 1935] only a few references regarding the use of alkali hydrolysis method were cited. Offering as they do an adverse comment on the use of this method, at least so far as *ragi* straw and bullock faeces are concerned, the findings reported in that paper [Seshan, 1935] make it necessary to examine in greater detail some more instances in point.

Sperry [1926-27] working on lipid excretion tested his control diets for their freedom from lipids by this alkali-hydrolysis method, the faecal lipids also being estimated by the same process. In brief, the method consisted in digesting 10 grms. of material for twenty-four hours with alkali over a boiling water bath, acidifying the digest with sulphuric acid, extracting with ether and taking up the resulting extract in petroleum ether for a subsequent drying to constant weight. It should also be noted that for a complete removal of the volatile fatty acids in the acidified digest, this was distilled to dryness under reduced pressure over a steam bath. In a similar experiment on the sterol metabolism of rats, Eckstein and Treadwell [1935] have, as a preliminary to the digitonin precipitation, separated the total lipids from the control diet as well as from the resulting tissues and faecal matter by digesting them with boiling 7·5 per cent alcoholic NaOH for two hours. Tidwell and Holt [1936] observe that a number of methods are available for the estimation of total lipids in faeces which depend on a primary saponi-

* For Part I, please see Vol. 5, P. 355 of this Journal.

fication of the material. Reference may also be made to the recent work of Horwitt, Cowgill and Mendel [1936] on a method of determination of the fat-content of spinach leaves. They attribute their failure to obtain results by directly saponifying vegetable tissues to a probable resistance offered by the cellulose structures of the plant.

It is apparent from the brief review given above that none of these workers considered the possibility of formation of a fresh quantity of fatty acids in the alkali digest of the material. Sufficient evidence has, however, been adduced in the previous paper to indicate such formation, under the conditions obtaining in the usual digestion with hot alkali, of samples of *ragi* straw and faeces. As this technique of fat-determination is of common use, it has been considered desirable to carry out further investigations to test its reliability and the results obtained are given in this paper.

Experimental

Repetitions of the alkali hydrolysis with *ragi* straw and faeces having yielded confirmatory results, a study of its effect on pure carbohydrates was undertaken. The experiments reported here were all conducted on pure specimens of cotton-wool, filter-paper, starch and dextrin as representing some types. It was thought advisable at the outset to determine the ether-extract content of these substances. Weighed quantities of the four samples in paper thimbles were subjected to a continuous extraction for eighteen hours in Soxhlet tubes. To test the completion of the extraction it was continued for another thirty-six hours. The results obtained were as follows :—

Table I

Control experiments with pure carbohydrates

Solvent	Percentage extraction				
	Period	Cotton-wool	Filter-paper	Starch	Dextrin
	Hrs.				
Petroleum ether B. P. 50°-60°C. . . .	18	0·025	0·025	*Nil*	0·050
Ditto . .	54	0·050	*Nil*	0·050	0·075
Ethyl-ether . . .	18	0·222	0·335	0·119	0·189
Ditto : .	54	0·192	0·285	0·149	0·189

The actual weights used for the extraction were about 2 grms. and the results were then calculated to a 100 grms. basis. So an error of one milligram in weighment would make a difference of 0·05 per cent. Although the extracts obtained

with petroleum ether show that the materials used were practically free from fat, the extraction with ethyl-ether yielded slightly different results. It is, however, sufficiently satisfactory for the purpose of this experiment to find that a continuation of the extraction over three times the period did not yield further quantities of extract.

Having thus determined the limits of the possible errors that might result from the impurity already present in the pure substances, they were subjected to the usual alkali hydrolysis treatment. The modified technique described in the first communication [Seshan, 1935] was followed. The method in brief was as follows :—

The substance, weighed out into a conical flask, was hydrolysed in aqueous 20 per cent alkali for twenty-four hours over a water-bath. The alkaline digest was then transferred to a distillation flask and rendered decidedly acid to litmus with sulphuric acid. The volatile acids, thus liberated, were steam-distilled and titrated against standard alkali. The mother liquid was rendered alkaline and retransferred to a porcelain evaporating basin. When the material was nearly dry, it was acidified with dilute hydrochloric acid and dried thoroughly over a water-bath, being powdered at the same time. The powdered material was then extracted in a Soxhlet. It might be mentioned here that the essential condition of this treatment with alkali, which alone matters for the purpose of this particular investigation, remains the same in this method as in those of others.

In this connection it is of interest to note that, in carrying out a volatile acid distillation, the condensation delivery-tube should dip well inside the receiving liquid, either water or standard alkali. By collecting the distillates in an open beaker in droplets, 500 c.c. of distillate gave varying titrations, with phenolphthalein as indicator, ranging from 0·5 to 3·0 c.c. N/10 alkali. This fact was repeatedly verified in distillations with pure solutions and hydrolysates as well, as will be seen from the data given in Table II.

TABLE II

Acidity given by 500 c.c. of distillate

Number of test	Blank mother-liquor with no carbohydrate in it c.c. N/10	Blank mother liquor with un-hydrolysed pure carbohydrates c.c. N/10
1	1·0	1·05
2	1·0	1·9
3	2·9	1·7

In every such case, the titration against alkali was quantitatively titrated back with standard acid. Apparently the variation was due to the carbon dioxide absorbed by the distillate.

After the first hydrolysis and ether extraction the residual substances were subjected to an identical treatment a second time, as was previously done in the case of *ragi* straw and faeces (*loc cit*). This resulted in a fresh yield of volatile and non-volatile extractives. The results obtained in the two treatments are presented in Table III.

TABLE III

Action of alkali on pure carbohydrates

Volatile acids and ether extractives expressed as per cent

No. of test	Nature of test	Analysis	Cotton-Wool	Filter-paper	Starch	Dextrin
First hydrolysis.	Steam distillation.	Volatile acids .	1·78	2·07	2·12	3·37
	Soxhlet extraction.	Non-volatile acids.	3·07	4·26	0·64	2·24
Second hydrolysis.	Steam distillation.	Volatile acids .	1·59	2·33	0·48	0·72
	Soxhlet extraction.	Non-volatile acids.	6·88	21·36	5·97	10·35

Evidently the appearance of these fatty acids is not due to the small amounts of impurities originally present. Control distillations were carried out and it was found that none of the four untreated carbohydrates gave any titration for volatile acids while the hydrolysis yielded large quantities. The increased yield of non-volatile fatty acids on a re-hydrolysis of the previously digested material also shows that the source of this fresh yield is none other than the pure carbohydrates themselves.

It is of interest here to note the composition of the non-volatile ether extractives which were formed by the action of alkali on these carbohydrates. In Table IV the analytical data are given :—

TABLE IV

Nature of the non-volatile ether extractives expressed as percentages

No. of test	Analysis	Cotton wool	Filter-paper	Starch	Dextrin
First hydrolysis .	Non-volatile ether extract.	3·07	4·26	0·64	2·24
	Acidity of above equivalent to c.c. N/10 alkali .	87·0	119·8	21·0	71·0
	Insoluble fatty acids. .	0·74	1·03	0·27	0·46
	Iodine value of above acids	31·5	27·6	44·2	38·8
Second hydrolysis	Non-volatile ether extract	6·88	21·36	5·97	10·35
	Acidity of above equivalent to c.c. N/10 alkali .	98·2	663·6	155·0	233·3
	Insoluble acids . .	2·65	3·42	1·18	1·42
	Iodine value of above acids	11·4	20·3	15·8	..

It was observed that the non-volatile extract was acidic in nature and that only a small fraction of it was insoluble in water. There was also a certain amount of unsaturated acid in the extract as shown by the iodine values recorded above.

Discussion

The main object of this investigation is to draw attention to the destructive nature of alkali hydrolysis on materials, which are largely carbohydrate in composition. The results recorded in the previous paper on the subject (*loc cit*) having shown the inadequacy of the method for fat-determination in cattle-fodder and faeces, it is of importance to know that the action of alkali has been proved to be exactly similar on pure carbohydrates. No attempt has been made here to find out whether all the substances that resulted from the hydrolysis were fatty compounds. But it has been shown that when substances are subjected to the treatment with alkali, which is an essential feature of the Liebermann process, volatile and non-volatile compounds are formed which can be separated by steam-distillation and are soluble in ether. It has also been shown that the treatment yields significant amounts of fatty acids of mixed composition.

N

This objection to the alkali hydrolysis method is, perhaps, a circumstance which should reasonably be anticipated from the elaborate researches of Nef [1907-14] on the action of alkalies on carbohydrates. In the monograph on Carbohydrates and Glucosides, Armstrong [1924] states that when carbohydrates are kept with alkali hydroxides at 37°C., the optical rotation of the solution decreases and the acidity increases. In reviewing the work of Nef [1907-14], he further adds that any carbohydrate in weak alkaline solution, in the presence of air or other oxidising agent, results in a mixture containing among several other compounds acids such as carbonic, formic, lactic, hydroxybutyric, hydroxyvaleric and hydroxyhexoic, as also some resins.

In conclusion, it may be stated that, where carbo-hydrate material is involved, the use of the Liebermann process of alkali digestion for fat-determination may appreciably vitiate the results obtained.

Summary

Previous work by the same author on the alkali hydrolysis method, as applied to *ragi* straw and faeces, having indicated a formation of fresh quantities of fatty acids, similar experiments were undertaken with pure carbohydrates, such as cotton, cellulose, starch and dextrin.

The results obtained were in complete accord with the findings previously recorded.

It is, therefore, suggested that whenever carbohydrate material is involved, the direct saponification of the tissue with alkali should be abandoned.

The author gratefully thanks Dr. F. J. Warth for his abiding interest in the work as also Dr. K. C. Sen, for his helpful encouragement.

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ABSTRACTS

Finding "Par" for dairy cows. NORDEN, E. C. (*Hoard's Dairyman*, **81**, No. 19, 492)

CHARLES STAFF, an animal nutrition authority, has prepared a series of standard production graphs for dairy cows as a result of fifteen years' accurate record keeping.

Exact records were kept of hereditary ability to produce milk, age, health and condition, supply of water, care and management, environment, weather conditions, character and quantity of the ration, date of freshening, and date of breeding for each cow.

By noting changes in these factors it became possible to handle these cows so as to obtain top production.

In arriving at a standard basis or 'par', all abnormal records were eliminated. In all, seventeen peak productions ranging from 25 to 65 lbs. were classified. The records were further classified according to date of breeding which governs the length of lactation period.

From a knowledge of peak yields, entire lactation yield and days in milk, standard milk-production graphs were made, for each set of data which proved true under practical conditions.

The amount of milk given at the peak of production is the guide or index for the group in which each cow belongs. The production curve of cows varies greatly according to their peak; the higher the peak production the faster the cow declines in milk flow and cows can be compared truly only by knowing their peak production and then comparing their performance on the identical days of their lactation periods. The records further indicate that early breeding, leading to a 300-day lactation period, is generally more profitable over a number of years. [C. E. M.]

Notes on feeding (mineral supply). CHARLES CROWTHER. (*J. Min. Agric.* (England) **43**, No. 10, 991)

THE author considers that the tendency to indiscriminate additions of minerals to rations has had detrimental effects in many instances; their proper use should be based on a knowledge of the mineral requirements of animals and the mineral content of food-stuffs. A balance between the individual mineral elements in rations should also be considered.

Any considerable excess of bases over acids or *vice versa* in a ration is undesirable; besides, interrelation between certain elements like Ca and P and Na and K should also be taken into account, and in working these the percentage figures for the various minerals must be corrected in proportion to the respective chemical equivalents for the element concerned.

Regarding what is the correct balance (or ratio) to aim at, the best temporary expedient to adopt is to take the mineral matter of milk as standard. In the light of such a comparison the mineral supplements most commonly needed are lime and salt.

High-yielding milch cows require 2 to 2½ oz. of finely-ground calcium carbonate per head per day, and growing cattle if they are not receiving a fair amount of good hay require 1 to 1¾ oz. of calcium carbonate. [C. E. M.]

The influence of the physical state of the fat on the calculation of solids from the specific gravity of milk. PAUL, F. SHARP and RAY, G. HART. (*J. of Dairy Sci.* **19**, 683)

THE previous temperature history of the milk influences its specific gravity at 15°C. This effect is due to variations in the physical state of the fat. The magnitude of the effect increases with the fat-content.

The determination of the specific gravity at 30°C., after previous warming to 45°C., for one-half minute is recommended as a method which will insure that the determination is made while the fat is in the liquid state.

The total solids are then worked out by the equation :—

Total Solids = 1·2537 Fat + 0·2680 Lactometer/Sp. Gr. of milk.

Variation in drying technique may in some cases account for the lack of agreement between the total Solids as determined by drying and as calculated from specific gravity.

Variations in lactose and citric acid and ratio of protein to ash content do not effect appreciably the reliabilty of the calculations of the total solids from specific gravity and fat-content of mixed milks. [C. E. M.]

The protein requirements of lactation. SAMUEL MORRIS. (*Nutri. Abstr. and Rev.* **6**, 273-280)

THE present review is mainly concerned with the practical aspect of the protein requirements of the dairy cow, the subject being discussed under the following heads : maintenance requirement, production requirement, effect of intake on yield, effect of quality of dietary protein and the value of non-protein nitrogen for lactation.

With any mixed ration, such as is commonly fed in dietary practice, 0·6 lb. digestible crude protein or 0·5 lb. digestible true protein per 1,000 lbs. live-weight is required for maintenance, the percentage utilisation of protein varying inversely with intake. With low intake the utilisation is high but unthriftiness and malaise occur, while with high intake the percentage utilisation is low, resulting in an unnecessary economic waste. About 70 per cent is considered as the best utilisation that can be expected under average practical feeding conditions.

If 1·2 to 1·3 times the protein in the milk be supplied as digestible true protein, the maximum level which will permit the highest possible milk yield will be achieved.

For the production of milk it is essential to supply in the food those amino-acids present in the milk protein which cannot be synthesised in the animal body. From the data given it is evident that certain food-proteins, *viz.*, proteins in the form of spring grass, silage from summer grass, low temperature dried blood meal, and pea-meal resemble milk-protein in their content of amino-acids more closely than do linseed cake, decorticated earthnut cake, maize or oats. For maximum milk production 0·5 lb. digestible true protein is required per 10 lbs. milk, but with proteins of high biological value, which supply all the essential amino-acids in the proper proportions for milk production, the amount does not exceed 0·44 lb. digestible crude protein per 10 lbs. of milk.

It is uncertain whether any part of the protein can be replaced by non-protein nitrogen. [N. C. D. G.]

The isolation of *Brucella abortus* from the milk of cows with negative blood reactions to the agglutination test. DOYLE, T. M. (*J. Comp. Path. Ther.* **49**, 320-327)

A LARGE number of samples of milk were tested from cows in Brucella-infected herds that failed to give an indication of infection on their blood test. Blood and composite samples of milk were collected from all cows under examination and serological tests of the blood were carried out in order to preclude the examination of milk from positive cases. Milk from each non-reactor cow was centrifuged and 2 c.c. of the cream and the deposit inoculated intramuscularly in two guinea-pigs. The milk whey was tested for the presence of brucella agglutinins. Cultures were made, on liver agar, from each spleen of guinea-pigs about the forty-fourth day after inoculation and incubated for ten days under 10 per cent CO_2 at 37°C. An agglutination test of serum from each guinea-pig was carried out. The antigen employed was made from a seventy-two hour old "smooth" non-thermo-agglutinable strain of *Brucella abortus* on liver broth culture and the density was adjusted by the addition of a forty-eight hour old washed liver agar growth. A water-clear whey was prepared by clotting centrifuged milk in the incubator to which rennet was added. The examination of 309 milk samples carried out by guinea-pig inoculation revealed the presence of *Brucella abortus* in samples from two cows whose blood proved negative on agglutination test. In one case milk from a positive case was tested from which the organism was isolated. One sample of whey yielded a titre of 1 : 100, but in this case the milk proved negative on guinea-pig test. With a positive blood titre of two test guinea-pigs the cultural examination of their spleens was negative. The author carried out serological tests of 684 cows during the course of this work and the average herd infection was found to be about 35 per cent. The possibility of the infection of the udder of cows with *Br. abortus* showing negative or low titre is discussed. [J. A. I.]

The breeding media of some common flies. THOMSEN, MATHIAS AND HAMMER, OLE. (*Bull. Ent. Res.*, **27**, 559-587)

THE observations recorded in this paper were carried out in Denmark, the two main species of flies concerned being the house-fly *Musca domestica* and the stable-fly *Stomoxys calcitrans*. Under natural conditions, the former was found to breed,

in order of preference, in fresh pig-dung, bedding of calf-boxes, horse-boxes and pig-sties, whereas horse-manure was of inferior importance in this respect. (These observations are of interest in view of the general belief that *M. domestica* prefers horse-manure to any other nidus.) As regards *S. calcitrans*, the larvae of this species were of infrequent occurrence in horse-manure, pig-dung and cow-dung, but they bred chiefly in the bedding of calf-boxes and to a smaller extent in humid remains of straw, etc., in corners of stables.

The authors also carried out a series of oviposition experiments to determine the relative attractiveness of pig-dung, horse-dung, calf-dung and cow-dung for gravid flies, these materials being exposed in jars placed in the open air, pig-sties and calf-boxes. In the case of *M. domestica*, little difference was observed between pig-dung and horse-manure in their power to attract ovipositing females, for both were highly attractive. On the other hand, calf-dung was much less favoured as a medium for egg-laying, while cow-dung did not contain any eggs at all. As regards *S. calcitrans*, it preferred horse-manure and calf-dung for egg-laying and only a small number of eggs were laid in pig-dung and cow-dung. From the results of these experiments and also from thier general observations, the authors conclude that "calf-stalls in practice have the greatest production of *Stomoxys* ". In discussing the possible reason why *S. calcitrans* rarely bred in horse-manure in manure-pits, while under experimental conditions they freely oviposited in this material, the authors point out that the high temperature produced by fermentation in large heaps of manure probably exercises a deterrent effect on ovipositing flies. As tor the absence of *Stomoxys* larvae even in manure in horse-boxes, the authors consider this as being due to the fact that stable-flies, as a rule, concentrate in cattle sheds, where they usually find suitable material for oviposition and are, therefore, not drawn to horse-manure. [S. K. S.]

Differential diagnosis in plant poisoning. STEYN, D. G. (*J. S. Afr. Vet. Med. Assn.* 7, 226-237)

IN view of the existing difficulty in arriving at a definite diagnosis of poisoning of which the author has had considerable experience in South Africa, an attempt has been made here to discuss some of the mineral and vegetable poisonings in which symptoms and post-mortem appearances, similar to those seen in infectious and other diseases, appear. The author has dealt, in a regular sequence, with the various diagnostic criteria as history of outbreaks, symptoms, post-mortem appearances and histology of lesions. It has been pointed out that a single toxic substance may produce peracute, acute, subacute or chronic poisoning depending upon the amounts ingested, and upon the conditions under which poisoning has taken place, thus giving rise to remarkable variations and even opposite effects on the animal system. That the symptoms and post-mortem appearances in heavy outbreaks of conical fluke infestation in sheep and trichostrongylosis in blackhead Persians may resemble to a remarkable degree those seen in cases of poisoning by gastro-intestinal irritants is mentioned.

While the fact of a large number of animals being taken ill or dying at the same time, severe purging and occurrence of death soon after the drinking of water (as in *Dichapetalum cymosum* poisoning) are suggestive of poisoning, the possibility of any microbic infection should invariably be eliminated by the microscopic examination

of blood and spleen smears. In outbreaks of infectious diseases, generally only a few animals take ill at the same time, and more cases follow later on, and fever is a frequent concomitant. Excepting cases of poisoning with gastro-intestinal irritants, fever is not usually seen in poisoning. All cases of reported sudden deaths are not actually so. Isolated cases of sudden death may take place, pointing to anthrax or poisoning or a large number of animals may die suddenly or within a short period.

Outbreaks may be (*a*) characterised by sudden deaths continuing to occur in a herd or flock for a prolonged period in the course of which very few or no animals are seen sick, or, (*b*) outbreaks of disease may set in with sudden deaths but in which symptoms are exhibited later during varying periods before death. Under group (*a*) anthrax, poisoning with *Vangueria pygmaea* (long period of latency sudden deaths continue for weeks in spite of grazing having been changed repeatedly), *Dichapetalum cymosum* (already mentioned above), and with hydrocyanic acid (mainly through eating wilted grasses) receive attention. A feature of *Vangueria* poisoning is that the highest mortality occurs in pregnant animals, and in those worked, while in chronic cases small greyish fibrotic areas are seen in the heart muscle, or the heart walls may be thin and fibrotic. HCN poisoning is associated with hoven, and is disclosed by chemical analysis of the ruminal contents. Sudden deaths may be caused by many other poisons, and by diseases, like peracute *lamsiekte*, heart water, anaplasmosis, etc. Regarding group (*b*), where outbreaks start with sudden deaths and symptoms develop in the later stages of their course, a large number of diseases are involved. Without dealing with them separately, the author passes on to the pathognomonic symptoms of various poisoning, including arsenic, cotyledonosis (torti-collis), crotalariasis (elongated hoofs), and poisoning with *Equisetum ramosissimum*, *Malva parviflora*, *Geigeria* (Vomiting), Senecio, strychnine, Tribulus (general icterus and photo-sensitisation), *Chrysocoma tenuifolia* (alopecia in kids and lambs).

Toxic agents and diseases with similar symptoms, and exhibiting similar post-mortem appearances are then discussed separately and in detail and this discussion should be consulted, in original, when required. Regarding symptoms of photo-sensitisation, it is stated that they may occur in many cases where the efficiency of the liver is decreased and where the animals have access to feeds rich in chlorophyll, and that these symptoms can be artificially produced by ligating the bile duct and supplying feeds rich in chlorophyll. [S. C. A. D.]

ORIGINAL ARTICLES

CROSS-BRED AND GRADE DAIRY CATTLE IN INDIA

BY

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(With Plates XVII-XX)

Cross-breeding

Cross-breeding means the crossing of different breeds; but the term, as generally used in India, refers to the mating of a European breed (*Bos taurus*) with the indigenous cattle (*Bos indicus*) of the country. It is in this restricted sense that the term is used in this article. Therefore, the cross-bred is an animal with 50 per cent European and 50 per cent indigenous blood.

Grading

Grading means the use of pure-bred sires either European or indigenous—on cross-bred or grade females.

Mating with the European sire is called "grading up" and with the indigenous sire "grading down".

Therefore, the grade is an animal with mixed European and indigenous blood, but not exactly 50 per cent of each. For practical purposes, it may be assumed that, if an animal has not less than 31/32 of the blood of one breed it is a pure bred.

F_2, F_3, Etc.

The use of cross-bred or grade sires is undesirable. The reason for this warning is because the cross-bred, or grade animal, owing to its ancestry, cannot produce a uniform set of germ cells, and therefore, cannot produce uniform progeny. Further, heterosis exhibited by the F_1 is not passed on to the offsprings. Although new breeds have been established by using cross-bred or grade sires, for example the Santa Gertrudis (3/8ths Zebu and 5/8ths Shorthorn), the establishment of such breeds requires time, skill in breeding and management, ample resources and courage to make drastic selections.

DANGERS OF CROSS-BREEDING AND GRADING

Successful cross-breeding, and subsequent grading, in adverse environments, when the difference between the milk-transmitting abilities of the parents is marked, requires a knowledge of breeding, restraint and skill in cattle management.

THE INFLUENCE OF CLIMATE ON MILK PRODUCTION

The influence that climate has on milk production is a subject of great interest to the dairy farmer in India, but it is too big a subject to be discussed fully in this article. Briefly, one of the obstacles to the production of heavy yields in tropical and sub-tropical climates is the heat, and the detrimental influence of heat is aggravated by increased humidity. The average annual mean atmospheric temperature and humidity are not sufficient data on which to judge the influence of these factors. The daily and seasonal deviations from these means are also important, because animals have great powers of recuperation if given a respite from the discomforts of unsuitable weather. Apart from the actual atmospheric temperature and humidity, the comfort of an animal during hot weather depends on the conductivity of the skin, the type of coat, the milk-yield, and the management. The conductivity of the skin, or the efficiency with which an animal can throw off the heat generated by its body, appears to be a breed characteristic. As a rule, animals indigenous to hot climates have this factor well developed. As would be expected, the heavier the coat, the greater the discomfort felt during the hot weather. Heavy coats are due mainly to heredity, but may be caused by a check in the growth curve due to mismanagement. The production of milk generates additional heat in the body in proportion to the yield. Generally, cross-bred and grade cows are not so efficient in throwing off body-heat as the indigenous animals, yet, on the average, their milk-yields are much higher. It is, therefore, not surprising that if such animals produce large quantities of milk during the hot weather, their constitutions, or the constitutions of their progeny, deteriorate.

Management can, to some extent, minimise the discomfort felt by milch cattle on account of the heat, but when animal shows signs of prolonged acute distress as indicated by a rise in temperature and rapid respiration, and receives insufficient relief during the night, then the only remedy is to decrease the strain, by reducing the milk-yield, or even drying off the animal.

A small amount of data collected at the Military Dairy Farms, Multan and Jullundur, bringing out the above factors, are given in Table I,

TABLE I

Statement showing the influence of heat on certain individual animals at the Military Dairy Farms at Multan and Jullundur. Temperature taken on 27th July, 1937.

Name of animal	Military Dairy Farm, Multan				Type of coat
Humidity 70 per cent	Breed	Daily milk-yield	Temperature of animal	Rate of respiration	Atmospheric temperature in shed-104° F. Time-3 P.M.
Hollyhock	Buffalo	26 lbs.	100·8	Normal	..
Flexseed	Cross-bred	25 lbs.	103·2	Very fast	Fine
Alacrity	Cross-bred	18 lbs.	103·0	Very fast	Heavy
Acup	7/8ths Grade	14 lbs.	103·2	Very fast	Very heavy
Mallogat	Indigenous cow	8 lbs.	101·2	Normal	Fine
Sumerun	Cross-bred	Dry	101·2	Normal	Fine
Adjunctive	Buffalo	Dry	100·4	Normal	..
Jimine	Indigenous cow	Dry	101·8	Normal	Fine
Humidity 89 Per cent	Military Dairy Farm, Jullundur				Atmospheric temperature in shed 92°F. Time-3 P.M.
Lackey	Cross-bred	40 lbs.	102·8	Fast	Heavy
Sheer	Buffalo	25 lbs.	101·8	Normal	..
Inder	3/4ths Grade	22 lbs.	102·8	Normal	Fine
Kamseen	Indigenous cow	14 lbs.	101·0	Normal	Fine
Lurgos	3/4ths Grade	Dry	101·6	Normal	Normal
Mianwali	1/4th Grade	Dry	103·0	Very fast	Very heavy
Mischief	Buffalo	Dry	100·2	Normal	..
Jenny	Indigenous cow	Dry	101·6	Normal	Normal

NOTE.—Multan has a very hot dry summer; at Jullundur the maximum temperature is usually lower but there is greater humidity.

Table I will be found instructive to breeders.

The buffalo is obviously the animal least influenced by atmospheric temperature and humidity. Hollyhock at Multan gave twenty-six pounds a day without discomfort, but Flexseed, a cross-bred, giving practically the same amount of milk in the same station, has a temperature of 103·2 and very fast respiration. Although the highest daily yield of an indigenous cow, in this table, is only fourteen pounds, it has been noticed that these animals are not, as a rule, distressed by heat, unless giving over twenty-five pounds of milk a day. It will be observed that except for Mianwali (1/4th grade) at Jullundur with an exceptionally heavy coat, all dry animals irrespective of breed were quite comfortable.

Table I indicates that if the dairy farmer, on the plains of India, is cross-breeding and grading and has to produce the same quantity of milk throughout the year, then a mixed herd should be maintained. Services should be so controlled that the majority of the buffaloes and average indigenous cows calve just prior to the hot season; and the majority of the cross-breds, grades, and high yielding indigenous cows, just before the cold weather.

Maximum limit of production

The production of milk imposes a certain strain on the constitution of an animal, the greater the quantity of milk produced, the greater the strain, and this strain is further increased in proportion to the adversity of the environment.

Although by cross-breeding and grading animals with improved milking capabilities may be bred, it is essential that these animals be given skilful management to relieve the strain on their constitution as much as possible. However, there are limits, both economical and practical, to the extent that skilful management may compensate for the additional strain of increased milk production. Therefore, in a given environment, there is a maximum limit of economic production for each animal which should not be exceeded. The more adverse the environment, the lower the maximum limits should be fixed. Without these limitations the dangers of cross-breeding and grading are increased in proportion to the difference between the ancestral environments of the sire and the dam.

Milk-yields that may be anticipated by cross-breeding and grading

Provided constitution is maintained, environment remains constant and one parent is a pure bred, it is possible to forecast the result of cross-breeding with reasonable accuracy.

In cross-breeding and grading, it may be assumed that the sire and the dam have potential transmitting abilities equal to the average of their respective breeds. Further, based on the genetic theory of intermediate, or blending inheritance, and the fact that breeds with marked dissimilar milk-producing abilities are being mated, it may be assumed that the offspring will have milk-producing abilities approximately half-way between the two parental breeds.

Working on the above hypothesis, the intermediate index for the cross-breds and different grades in Table II has been compiled.

TABLE II

Comparison of the intermediate index and the actual lactation yield of different breeds

Breeds	Intermediate index lbs.	Actual lactation yield lbs.	Error ± Per cent
Pure Friesians (European)	9,400	9,406	..
7/8ths grade	8,700	7,076	—18·7
3/4ths grade	8,000	6,494	—18·8
5/8ths grade	7,300	7,224	—1·0
Cross-bred	6,600	6,882	+4·3
1/4th grade	5,200	5,250	+1·0
1/8th grade	4,500	4,123	—8·1
Sahiwal (Indigenous)	3,800	3,789	..

NOTE 1.—The actual yields have been taken from Table III. Lactation yields have not been corrected for days in milk because it is considered that, with a large population, actual yields give a true indication of a breed capabilities.

NOTE 2.—The intermediate index of the different breeds has been worked out by rounding off the yields of the pure Friesian, and the Sahiwal to the nearest hundred and then assuming that the intermediate index of the other breeds would be approximately half-way between the two parental levels.

EXAMPLE 1.—The intermediate index of the 1/4th grade which is produced by crossing a Sahiwal bull on a cross-bred cow would be $\frac{3800 + 6600}{2} = 5200$.

EXAMPLE 2.—The intermediate index of the 5/8ths grade which is produced by crossing a Friesian bull on a 1/4th grade cow would be $\frac{9400 + 5200}{2} = 7300$.

A study of this table is instructive. It will be observed that in most cases the theoretical intermediate index agrees fairly closely with the actual yield.

The larger errors are explainable. The cross-bred due to heterosis, or hybrid vigour, shows the biggest plus error. The strain on the constitution of the cross-bred is reflected in its graded-up progeny, and the 3/4ths have a minus error of 18·8 per cent. The graded-up progeny of the 3/4ths do not recover constitution and the actual yield of the 7/8th still remains 18·7 per cent below the intermediate index. By grading down from the cross-bred to the 1/4th the actual yield drops 1400 lbs. but constitution is regained and the lower actual yield is one per cent above the intermediate index. By grading up from the 1/4th with regained constitution, the actual yield of the 5/8ths is only one per cent below the intermediate index.

The reason why the 1/8th did not do better is explained perhaps by the lack of ability, even amongst most strictly selected indigenous bulls (which are not genetically pure breds) of transmitting a uniform milk-producing ability. The population is, perhaps, insufficient to disguise this lack of uniformity.

It may be taken, as a rule, that to regain constitution the breeder should grade down, and to regain milk grade up.

Choosing the sires

European Sires.—For economical and physiological reasons, it is the general practice to cross-breed and grade-up by means of European bulls.

The problem of which breed of European bull to use is important. Once having decided on a breed it is advisable—unless the choice is absolutely wrong—not to change. The fewer the divergent characteristics in a grade, the easier it is to arrive at accurate conclusions about the success or failure of a particular breeding policy for a particular environment, and to make any alteration or modification that may be necessary.

The main object of cross-breeding and grading in India is to improve on the milk-producing ability of the indigenous cows. As the milk of these animals has a comparatively high butter-fat content, the possibility of reducing this characteristic is of minor immediate importance. Again to increase the yield, means increasing the strain on the constitution, therefore, the constitution of the progeny must be sound. Although calf-management plays no little part in the final constitution of the adult animal, the average size and robustness of the European sire is also important. The length of the coat, the quality and colour of the skin, are factors which influence the comfort of an animal during the hot season, especially if producing milk. Therefore, what is required is a sire with a large frame, robust constitution, smooth coat, dark skin, preferably, and the ability to transmit the capacity to produce large yields to his progeny, as indicated by his breed average.

Because the European bull, again for economical and physiological reasons, should be imported when young, preferably nine to eighteen months; it will not be possible to obtain any index as to his transmitting abilities. Reliance will have to be placed on his breed, his pedigree, the average worth of the herd from which he is purchased, and the ability and integrity of the breeder.

Although it is not economical, to pay a fancy price for a young European bull to be used for cross-breeding, or grading, it is also not advisable to purchase a European bull merely on price.

There is no one European breed best suited for cross-breeding and grading on all the different indigenous breeds in India, but probably the breed giving the most general satisfaction is the Friesian.

Good bulls of this breed with a probable index of 9,000 to 10,000 pounds can be purchased for Rs. 1,000 to Rs. 1,500 landed in India.

Indigenous sire.—The indigenous sire should be of the same breed as the original indigenous dam, and the best procurable. If available, the pedigree should be traced back to the great-grand parents to be sure of the purity of blood and that dairy qualities are present in the ancestry. The better the indigenous bull, the less the drop in milk-yields from grading down. If no reasonably good pure-bred of the same breed as the indigenous dam is available, then it is better to introduce another indigenous breed than to grade down with an inferior sire.

Choosing the original dams

Because the milking capabilities of cross-breds are approximately half-way between the two parental levels, it is advisable to start off with dams of the best milking strains available. Due to the importance of constitution to a deep milker, and further as constitution appears to be transmitted from the dam to its offspring, the dam must have a good constitution. Seldom will a weedy indigenous cow produce a really successful cross-bred, and certainly not a cross-bred from which to grade up. If the local cows have poor constitutions, it would be advisable by improved management and breeding to produce constitutionally sound indigenous dams, before attempting to get higher yields by cross-breeding and grading.

It is preferable that the indigenous dams should belong to recognized dairy breeds and be typical specimens of their breed, so showing signs of reasonable purity.

Actual results obtained by cross-breeding and grading by the military dairies in the northern circle

The history of the importation of foreign sires by the Military Farms Department, to cross on the indigenous country cows, dates back to about 1900. However, it is only within the past fifteen years that importations have been made in sufficient numbers to influence, appreciably, the herds owned by this Department, and only within the past decade that the question has been thoroughly investigated and reliable statistics collected.

It was realized that data to be reliable must be based on sufficient population, and to obtain comparable data on a sufficient population, the controllable environmental factors must be standardized at all farms. The following factors were standardized :—

(1) European breed to be imported.

(The Friesian was considered the most suitable).

(2) Feeding of—
  (a) Young stock.
  (b) Dry stock.
  (c) Milking herd.
(3) Number of milkings.
(4) Dry periods.
(5) Service periods.
(6) Extra ration for climatic conditions.
(7) Mineral feeding.
(8) Accommodation.

There were certain factors such as climate, quality of fodder, water, etc., which could not be made equal at all farms, but it was considered that by spreading the breeds over different farms the variations in the above factors would be smoothed out.

The data given in Table III is based on all the lactation yields of the different breeds noted, for the past two years.

TABLE III

Statement showing the average lactation yield of various breeds on Military Dairy Farms in the Northern Circle dried off during the period 1st April, 1935 to 31st March, 1937

Breed	Population	Average lactation yield	Average days in milk
		lbs.	
Pure Friesian	11	9,406·5	239·1
7/8ths grade	30	7,076·7	323·4
3/4ths grade	290	6,493·7	317·9
5/8ths grade	86	7,223·7	325·5
Crossbred	572	6,881·7	318·2
1/4th grade	225	5,249·7	318·5
1/8th grade	34	4,123·2	295·7
Pure Indigenous	429	3,798·1	281·0

DEATHS AND CASTINGS

In comparing the economic value of different breeds it is not sufficient to study the milk-yields only, it is necessary also to have data on the number of castings and deaths. Because animals are cast when it is uneconomical to keep them in the herd, every animal cast tends to increase the average efficiency of those remaining. In conjunction with castings, deaths must be taken into account. Unless the longevity of an animal, under the strain of producing extra milk, is maintained, the production of this extra milk may be unsound economically.

TABLE IV

Table showing the castings and deaths amongst the adult and young stock cows of the Military Dairies in the Northern Circle during the years 1st April 1935 to 31st March 1937

Particulars of casualties	Breeds—Adults								Breeds—Young stock							
	Friesian	Grades			Cross-bred	Grades		Indi-genous	Frie-sian	Grades			Cross-bred	Grades		Indi-genous
		7/8	3/4	5/8		1/4	1/8			7/8	3/4	5/8		1/4	1/8	
Population	12	44	279	119	479	151	32	320	10	150	346	137	283	38	18	103
Cast for low yield	...	...	2	...	3	11	...	38	...	...	...	...	...	...	...	...
Cast for old age	...	...	3	1	14	2	...	8	...	...	...	...	...	...	...	...
Cast for sterility	...	...	8	2	18	7	1	59	...	...	4	4	1	1	...	10
Cast for physical defects	...	2	5	1	6	4	...	15	...	5	4	2	5	...	...	2
Cast for udder trouble	...	...	1	...	4	3	1	7	...	...	...	...	...	...	...	...
Cast for misc. reasons	...	...	6	1	15	5	2	11	...	4	5	2	2	3	1	7
Deaths	1	6	39	12	46	10	4	21	...	31	60	18	28	4	4	9
Total casualties	1	8	64	17	106	42	8	159	...	40	73	26	36	8	5	28
Percentage of deaths to population	8·3	13·4	13·9	10·1	9·6	6·6	12·5	6·6	...	20·7	17·3	13·1	9·9	10·5	22·2	8·7
Percentage : Total casualties to Population	8·3	18·1	22·5	14·4	12·1	27·9	25·0	49·7	...	26·7	21·1	19·7	12·4	21·0	27·8	27·2

NOTE.—Population figures denote the average daily number of each breed present during the period.

Table IV is instructive. If breeds with a population of less than 40 are ignored—as such data may be unreliable—it will be observed that there is a tendency for the percentage of deaths to population to increase with the increase of European blood. However, because the lower grade and indigenous animals have a bigger percentage of castings, the total casualties in the different breeds indicate that the higher grades can compete with the other breeds in their length of time in the herd, which is the important economic factor.

The castings amongst the indigenous stock is heavy, and this factor combined with low yields (Table III) reflects on the economic worth of these animals, even when specially selected and belonging to the best milking strains available.

A tendency to sterility is one of the most costly faults that a milking breed can possess. Amongst indigenous animals 18·4 per cent of the adults and 9·7 per cent of the young stock were cast for this defect. These percentages compare unfavourably with the averages of the other breeds which work out to 3·2 per cent and 1 per cent respectively.

Generally, Table IV indicates that, in India, the breeder need not fear excessive losses from castings and deaths amongst cross-bred and grade cattle, provided that other adverse environmental factors are compensated by good management. However, it may prove more profitable to maintain indigenous stock, despite their many uneconomic characteristics, rather than breed cross-bred and grade animals in an environment too adverse, or with management too inadequate, to compensate for the strain of increased efficiency.

Figures

Eight figures in Plates XVII-XX are photographs of some typical cows of the different pure-breeds, cross-bred and grades mentioned in this article. The gradual change in conformation, with the increase in European blood, is clearly seen.

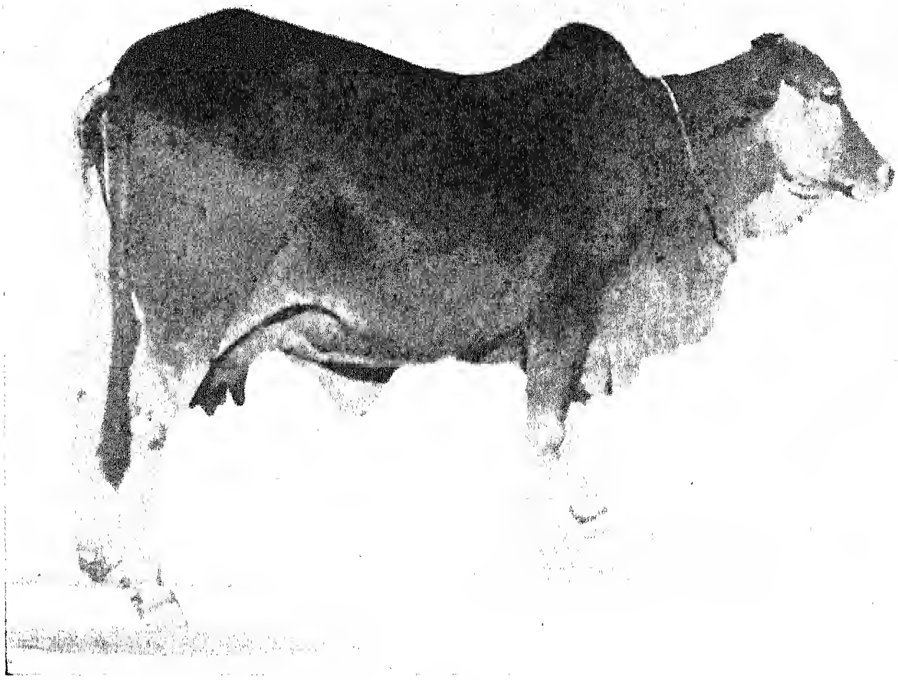

FIG. 1. FEROZEPORE MANIHARI

A typical Ferozepore-type Sahiwal. Yielded 6,954 lbs. of milk in 327 days in her first lactation. In the second lactation gave 8,837 lbs. in 272 days and still yielding 23 lbs. a day.

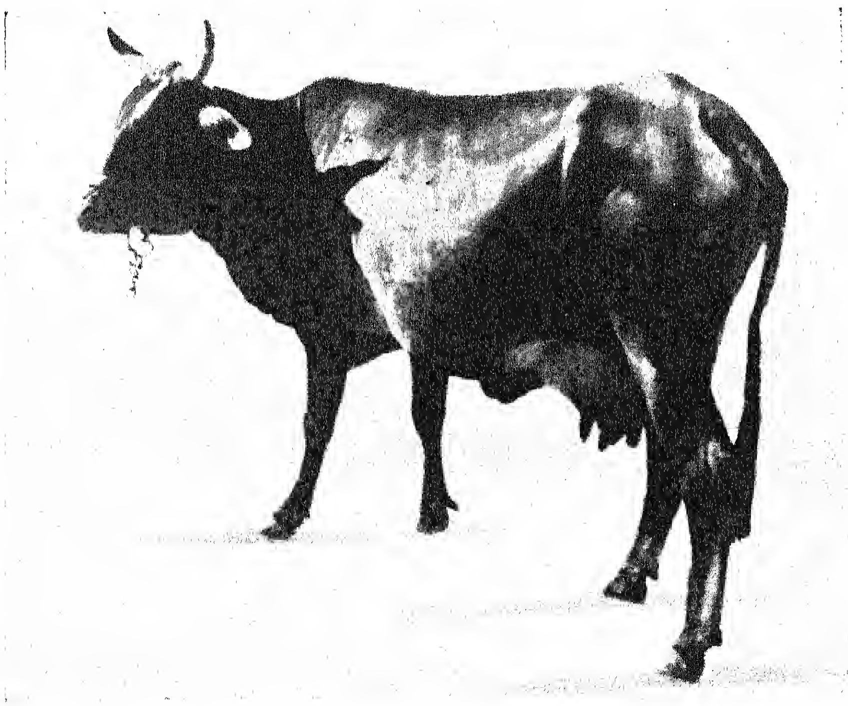

FIG. 2. GAMBAR FURSAT

A typical one-fourth grade in conformation. Yielded 7,580 lbs. of milk in 406 days.

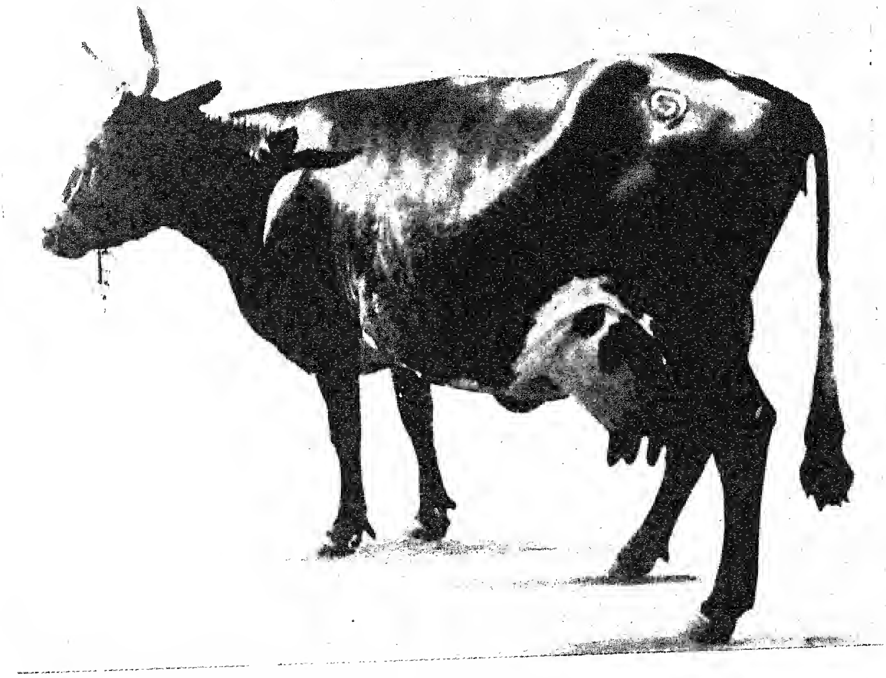

Fig. 1. SARGODHA DODY

A cross-bred. Best yield 13,180 lbs. in 350 days.

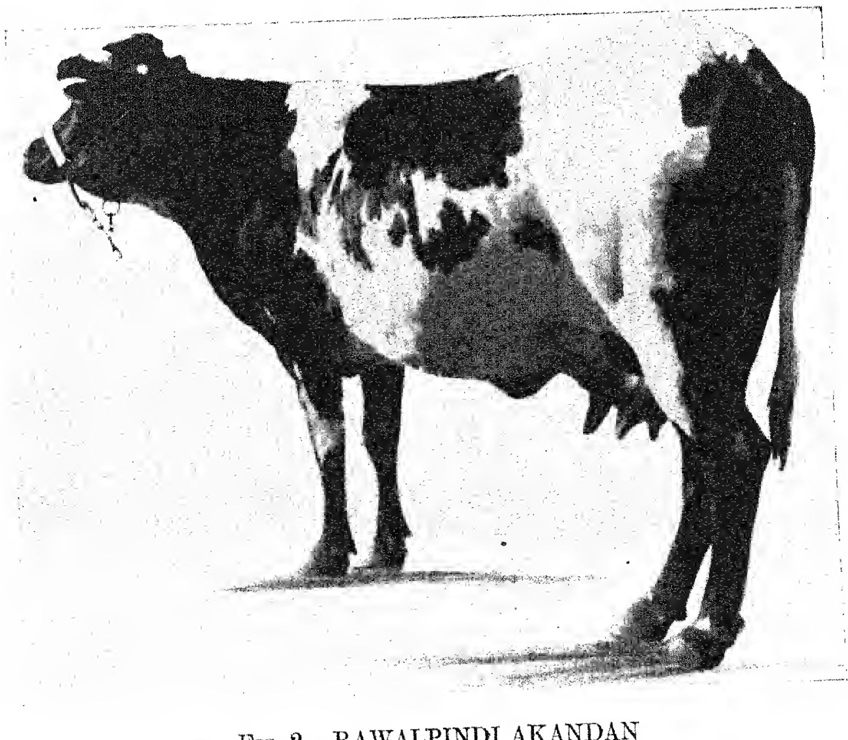

Fig. 2. RAWALPINDI AKANDAN

A five-eighths grade. Best yield 9,430 lbs. in 317 days.

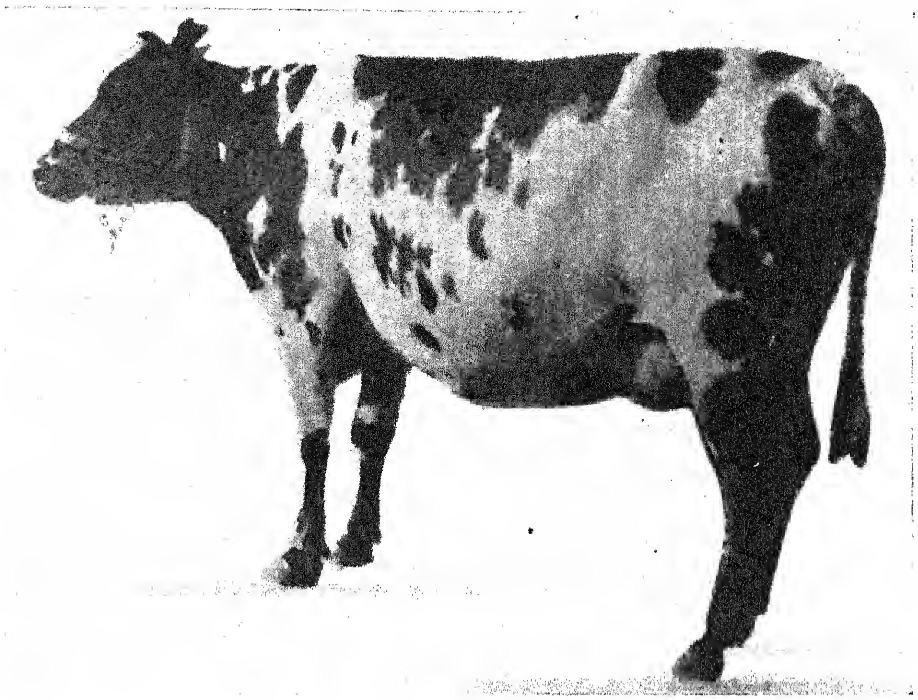

FIG. 1. ANOOP KALI
A three-fourths grade. Best yield 10,561 lbs. of milk in 340 days.

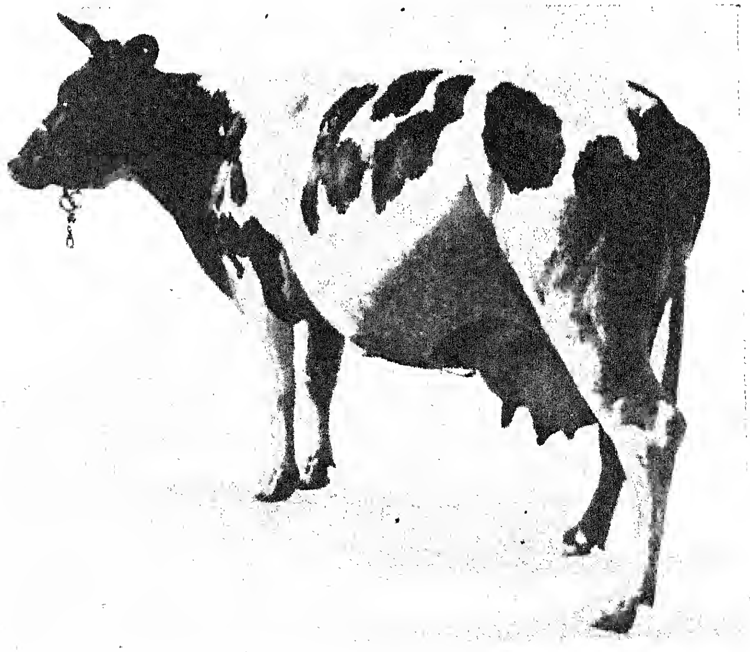

FIG. 2. RAWALPINDI ABBEY
A seven-eighths grade. Best yield 10,200 lbs. in 281 days.

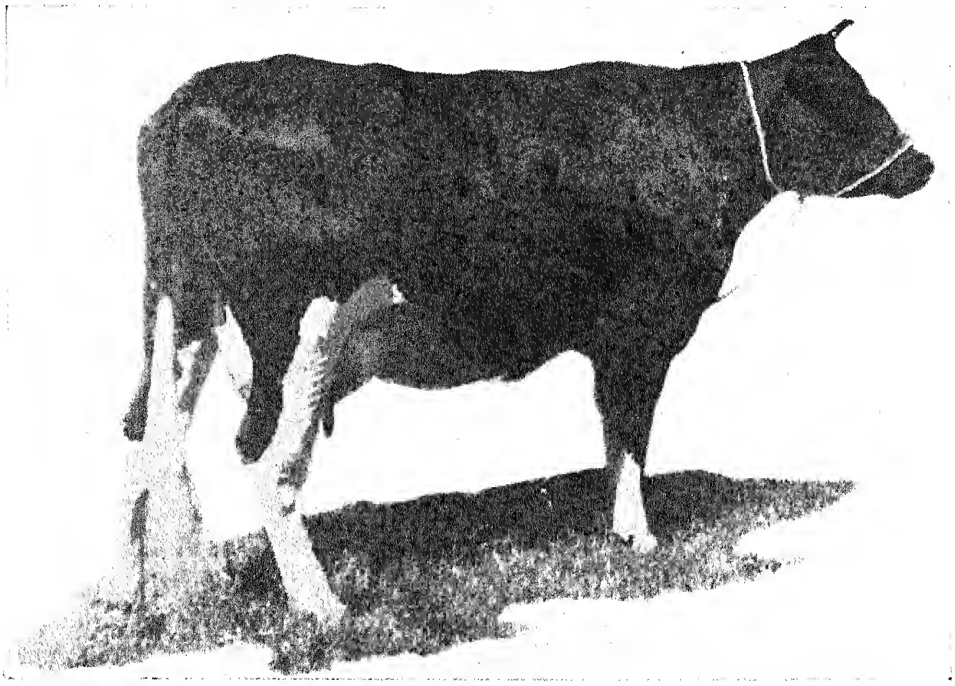

Fig. 1. RAWALPINDI ALLEGBAU

A Friesian cow bred in India. Gave 7,448 lbs. in 212 days at the Military Dairy Farm, Sialkot. Dried off to prevent strain of milk production during the hot weather. Shows plenty of constitution.

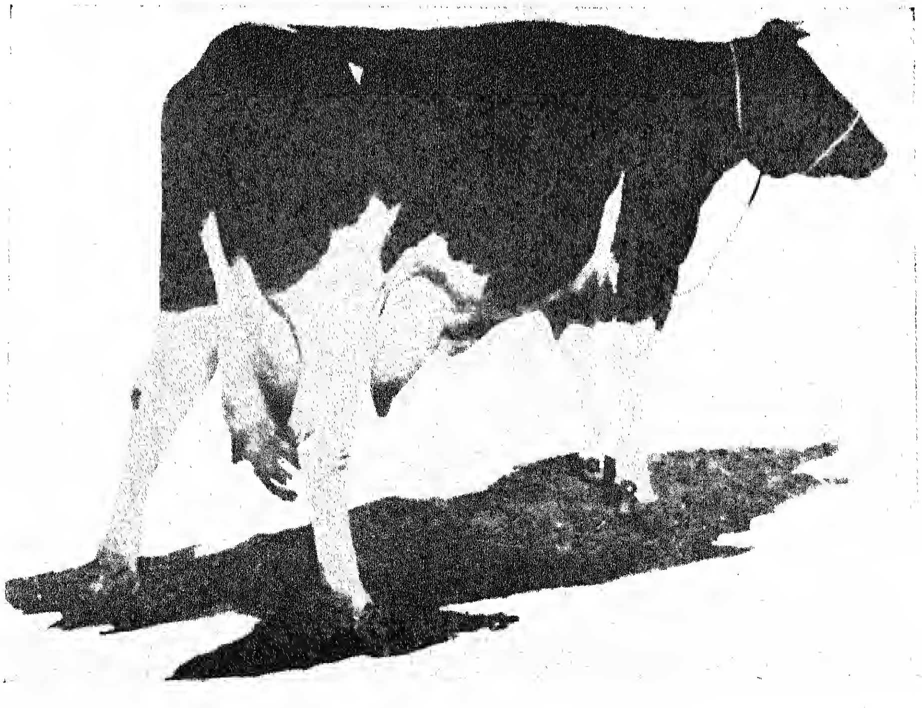

Fig. 2. RAWALPINDI BERDRAGA

Another Friesian cow bred in India. Gave 10,188 lbs. milk in 211 days at the Military Dairy Farm, Sialkot. Dried off to prevent strain of milk production during the hot weather. Shows plenty of constitution.

ON THE TREATMENT OF *BABESIA BIGEMINA* INFECTION IN CATTLE IN INDIA

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THE introduction of trypanblue in the field of therapy of red-water in cattle, due to *Babesia bigemina* infection in cattle, was the result of pioneer work carried out by Nuttall and Hadwen [1909]. Trypanblue has ever since been used almost universally as a remedy against this infection. In India, cattle are known to be infected ubiquitously with various protozoan parasites which ordinarily reside in a latent state in the tissues of the hosts and cause no manifest disturbance. It is when the restrain on such parasites is relaxed by the onset of some febrile diseases such as rinderpest that they multiply at such a rate as to cause serious disease and frequently death in the affected animals [Edwards, 1927]. Trypanblue, as would appear, is rarely called into use in clinical cases of piroplasmosis in cattle as a primary affection in this country, due to the fact that most of the animals are 'carriers', and it is usually in cases of resuscitation that treatment with drugs is resorted to.

In this Institute where a large amount of work on rinderpest is carried out, the control of *Babesia bigemina* with trypanblue presented serious obstacles in recent years. The rinderpest bull virus, which is always maintained in the Institute in successive batches in hill bulls, was found to be more often than not, contaminated with the red-water parasites rendering it unsafe for issue in the field. The mortality in such complicated cases occurred to the extent of 59·3 per cent in 1933, 55·5 per cent in 1934 and 40·7 per cent in 1935, as compared to 25·6 per cent, 16·8 per cent and 23·2 per cent during the same period respectively in uncomplicated cases. Apart from this, in the tests carried out to evaluate the potency of anti-rinderpest serum that is manufactured at this Institute, very misleading thermal reactions were set up due to the resuscitation of *Babesia bigemina*. In such cases the usual dose of $\frac{1}{2}$ to $\frac{3}{4}$ grm. of trypanblue was administered intravenously in a 1 per cent solution per an approximate weight of 100 kg., but in a number of cases the parasites were found to be persisting, resulting in death in several cases. Due to a large number of such failures encountered

in the treatment of *B. bigemina* infection, with the popular drug trypanblue, work was taken in hand early in 1934 to evolve more effective measures for the control of these organisms. A series of controlled experiments were carried out to test the efficacy of trypanblue in larger doses on the laboratory strain of *B. bigemina*. The strain was isolated and maintained in successive batches in hill bulls, but as would appear from the data collected, the drug failed to check the multiplication of the parasites. On perusal of the original work of Nuttall and Hadwen [1909], it would appear that although trypanblue produced a marked amelioration of the symptoms and checked the severity of the disease, yet in two out of the five treated cases, the parasites reappeared in the peripheral blood on the 7th and the 8th day respectively after the treatment. Theiler [1912] working in Pretoria on the same lines infected twelve susceptible cattle with the local strain of *B. bigemina* and treated them with trypanblue. Except one animal that died, all recovered from the disease. In three of his treated cases, however, microscopic examination of the peripheral blood revealed the presence of the parasites on 14th, 18th and 21st day respectively after the treatment.

A fresh stock of trypanblue was obtained from the manufacturers, E. Merck & Co., Germany, and comparative potency tests were carried out along with the old stock but in the experiments carried out no evidence could be obtained of the superiority of the new stock as compared to the old. The drug was subsequently replaced by another proprietary preparation ' Akiron R ', manufactured by Bayer & Co., with very successful results.

METHODS, MATERIAL AND OBSERVATIONS

At the outset work was undertaken to determine the maximum tolerable dose of trypanblue in the hope of combating the infection due to *B. bigemina* with a higher dose rate. Sergent and co-workers [1927] advocated small doses in proportion to the number of parasites present in individual cases, as they were of opinion that one grm. of trypanblue per 100 kg. is liable to cause alarming symptoms due to the liberation of toxins from the destroyed parasites. But the treatment carried out at this Institute, with 50-75 c. c. of a 1 per cent solution given intravenously, failed to cleanse the peripheral circulation in several cases.

For the purpose of M. T. D. tests of trypanblue, fourteen animals were injected intravenously at doses varying from 1·2 to 4 grms. in a 2 per cent solution at an average body-weight of 220 lb. Very interesting observations, some of which have been recorded by Stirling [1927], were made which are worthy of mention.

In almost all the cases, the inoculation of trypanblue was followed within a short period, depending on the degree of the dose used, by accelerated breathing, filling in of the folds of the skin round the eyes and the neck, lachrymation and

salivation. The tears and the saliva were bluish in colour, and discolouration of the conjunctival and the buccal mucous membranes soon followed. The non-pigmented parts of the skin, over the regions of the groin and scrotum were stained blue a little later. A marked tendency towards defecation was observed and on straining, transparent jelly-like mucus was passed. The inoculated animals appeared to be depressed and made no attempt to rise soon after the injection. The symptoms passed off within two hours in animals that received smaller doses.

Donatien and Lestoquard [1927] are of opinion that the injection of trypanblue into healthy or chronically infected cases does not give rise to toxic symptoms but on the other hand believe that such symptoms are due to the liberation of toxins on account of lysis of the parasites. Contrary to the view expressed by the above-mentioned workers it may be pointed out that the symptoms described above were observed in the batch of fourteen bulls used for M. T. D. tests that may be classed as healthy or chronically infected in view of the fact that none of these animals showed evidence of red-water when they were subjected to the experiment. In those animals that received the maximum dose, the drug was noticed to be eliminated with the body excretions six minutes after the inoculation; while in others that received a smaller quantity this process took from ten to thirty minutes. Severe toxic symptoms were exhibited on inoculation of 4 grms. of trypanblue, *viz.*, depression, loss of control over the limbs, diarrhœa, inappetence and dyspnœa. Tympanites is not an uncommon symptom that is associated with the introduction of trypanblue. The discolouration of the mucous membranes and tissues have been noticed to persist over four months. As a result of these observations the maximum dose of the dye that could be tolerated with safety was determined to be between 3 to 3·5 grms. per 220 lb. body-weight. In order to test the efficacy of this heavy dose of trypanblue, four healthy animals were artificially infected with *B. bigemina.* Two of these were treated with trypanblue, one with three successive injections of 3·5, 2·5 and 1·5 grms. and the other with two consecutive doses of 3 and 1·5 grms. on 5th and 6th day after the infective inoculation, respectively. The remaining two animals were untreated to serve as controls. All four animals were then infected with rinderpest virus on the 10th day after the initial inoculation. In both the treated cases the parasites disappeared after two and three days following the last injection of trypanblue respectively, but on infection with rinderpest, however, *B. bigemina* reappeared in both these animals on 10th and 9th day respectively, and persisted until death in each case. In the two controls, *B. bigemina* became more frequent on infection with rinderpest and in one case persisted until death. In the other case they disappeared after 21st day and the animal survived. From this experiment it will be observed that inspite of the attempt to cleanse the circulating blood with large doses of trypanblue, the parasites flared up as a result of infection with rinderpest and, curiously enough, brought about death in both the treated animals.

In a further series of experiments the effect of administering trypanblue immediately before a combined infection with rinderpest and *B. bigemina* was tested. Two healthy bulls were given 3 grms. of trypanblue each, in a 2 per cent solution intravenously followed by *B. bigemina* infected rinderpest virus a little later. Two controls were inoculated with the virus only. All the four animals showed organisms ten to twelve days after inoculation as trypanblue produced no effect whatever in the treated cases. All four animals, however, lived, and were discontinued when the parasites had disappeared from their circulation.

Notwithstanding the disappointing results obtained in the foregoing experiments in the treatment of the tropical red-water parasites by means of trypanblue, it was thought that these failures may be attributed to a deterioration in the potency of the drug used owing to long storage, or to the possibility of the particular strain of the parasites that was being used, having developed a tolerance to trypanblue as a result of continuous passaging through animals treated with this drug. The strain of this piroplasm was isolated and maintained in a series of successive passages in hill bulls that had been discontinued from rinderpest work. Only such animals were selected for this purpose as had not exhibited *B. bigemina* previously. The strain was successfully established in these animals throughout nine passages, using two animals each time. The sub-inoculaticns were done with blood drawn during paroxysms, about six to seven days after infection. Although none of the animals developed clinical symptoms or showed marked thermal reaction inspite of about thirty to forty per cent of the erythrocytes having been infected, yet only one animal proved refractory. Three animals showed the parasites seventy-two hours after sub-inoculation, eight animals on the 4th day, three on the 5th, one on the 7th and one on the 11th day. In each case the number of the parasites gradually decreased after a phase of increased multiplication without producing any ill effects.

Treatment with a fresh stock of trypanblue, obtained from the manufacturers, E. Merck & Co., Germany, was now taken up with a view to test its comparative potency with the old stock. In all, eleven animals were treated as tabulated below :—

TABLE I

Table showing the results of treatment of B. bigemina *with fresh and old trypanblue*

No. of animal	Date of infection	Date of treatment	Stock of Trypanblue	Results	Remarks
Passage X. Hill Bull 484.	16th April '35	20th April '35	Fresh trypanblue.	Showed parasites for 15 days after treatment.	Severe toxic symptoms exhibited.
Passage X. 341	16th April '35	22nd April '35	Old trypanblue	Showed parasites on 4th day after treatment.	No toxic effects produced.
Passage X. 276	16th April '35	Untreated Control.	...	Showed parasites throughout the period of observation.	...

TABLE I—*contd.*

No. of animal		Date of infection	Date of treatment	Stock of Trypanblue	Results	Remarks
Passage XI.	Hill bull 111	23rd April '35	28th April '35	Fresh trypan-blue.	Showed parasites for 5 days after treatment, reappeared on 15th and 17th day.	No toxic effects produced.
	386	23rd April '35	28th April '35	Old trypan-blue.	Showed parasites occasionally during the period of observation.	Severe toxic symptoms exhibited.
	213	23rd April '35	Untreated Control.	...	Showed parasites throughout the period of observation.	...
Passage XII.	699	30th April '35	3rd May '35 .	Fresh trypan-blue.	Showed parasites throughout the period of observation.	Slight toxic symptoms exhibited.
	647	30th April '35	3rd May '35 .	Old trypan-blue.	Ditto	Ditto.
	166	30th April '35	Untreated Control.	...	Showed parasites throughout the period of observation.	...
Passage XIII.	198	8th May '35	17th May '35	Fresh trypan-blue.	Showed parasites for 3 days after treatment.	No toxic effects produced.
	517	8th May '35	Untreated	...	Reacted late (15th day).	...
	623	8th May '35	Untreated Control.	...	Showed parasites throughout the period of observation.	...
Passage XIV.	591	15th May '35	21st May '35	Fresh trypan-blue.	Showed parasites throughout the period of observation.	No toxic effects produced.
	594	15th May '35	20th May '35	Old trypan-blue.	Ditto	Ditto.
	295	15th May '35	Untreated Control.	...	Showed parasites throughout the period of observation.	...
Passage XV.	584	24th May '35	28th May '35	Fresh trypan-blue.	Showed parasites on the 2nd day after treatment.	Slight toxic symptoms exhibited.
	334	24th May '35	28th May '35	Old trypan-blue.	Showed parasites throughout the period of observation.	Ditto.
	407	24th May '35	Untreated Control.	...	Showed parasites throughout the period of observation.	...

It would appear from Table I that six animals were treated with the fresh stock of trypanblue and five with the old, leaving seven animals untreated to serve as controls. In all cases treatment was instituted on the day the parasites were detected in their blood after an incubation period of three to six days. On the basis of M. T. D. tests carried out at the commencement of this work, all the animals under treatment were given 30 c.c. of a 1 per cent solution of trypanblue per 25 lb. body-weight intravenously. Goodall [1914] reported successful results with heavy doses of trypanblue up to 250 c.c. of a 2 per cent solution intravenously for cattle infected with *B. bigemina* and attributed failures to be due to small doses being used. But our results did not support his views as in none of the eleven cases so treated was the sterilization of the circulating blood obtained during the post-treatment period of observation of twenty days.

The above series of experiments yielded almost conclusive evidence as to the ineffectiveness of trypanblue to be regarded as a 'specific' for the tropical red-water parasites. Several eminent workers have used trypanblue extensively in the treatment of bovine piroplasmosis due to *B. bigemina* infection doubtless with amelioration of the symptoms but, in several instances their records either do not show that the diagnosis was based on microscopical examination, or that the cures were established on examination of blood films after the treatment. Thus, Mellis [1914] reported to have treated twenty-three cases with trypanblue in North Africa on clinical manifestations and obtained nineteen recoveries. In the four cases that died the drug was stated to have been given subcutaneously. Goodall [1914] reported to have treated hundreds of cases with large doses of trypanblue but the effect of the drug was not determined by microscopical examination. Yakimoff and collaborators [1917] used 25 c.c. of a 1 per cent solution in normal saline giving a total of 2—3 grm. in acute cases. While stating that the cures were 'complete', the authors do not bear out this on blood smear examination.

The possibility of the strain having acquired drugfastness was also ruled out as a result of similar tests being carried out with an untreated strain of *B. bigemina* obtained from the field. For the purpose of this work seventeen healthy animals were artificially infected with the field strain. Five of these were treated with the old stock of trypanblue and six with the fresh, leaving six to serve as controls. Only one animal proved refractory to the infection. The treatment was carried out under identical conditions as in the case of the first lot of the laboratory strain of *B. bigemina*, employing the same dose rates of the two stocks. The results obtained in this case could lend no support to the presumption that the untreated strain of the parasites was amenable to treatment with either brands of trypanblue. On consideration of the results, the use of trypanblue had, therefore, to be discontinued. Recently, Theiler [1930] expressed the view that since the war substances called trypanblue have been produced but they are devoid of parasiticidal action.

About this time a communication was received from Havero Trading Company, Calcutta, offering samples of a proprietary preparation, 'Akiron R', manu factured by Bayer & Co., for trial in 'Babesiosis' and 'Theilerioses' of cattle. At that time no reference could be obtained in available literature regarding the therapeutics of this drug, on which experiments could be based, but the opportunity was taken and the drug obtained free of charge. It was received in 5 and 10 c.c. ampoules of a 5 per cent solution, the fluid being of a light yellow colour and ready for use. The original laboratory strain of *B. bigemina* was maintained and in the subsequent series of experiments the efficacy of 'Akiron R' was tested with very successful results. These have been tabulated below :—(Tables **II-A** and **II-B**).

TABLE II-A

Showing results of treatment of B. bigemina *infection with 'Akiron R.'*

No. of animal		Date of infection	Date on which parasites appeared	Date of treatment and dose	Results of blood smears	Remarks
Passage XVI.	Hill bulls 246	31st May '35	12th June '35 Δ *B. big.*	12th June '35, 10 c.c. sub-cutaneously.	Negative following day till the period of observation.	Thermal reaction for 48 hours, inappetence, local swelling lasting for a week, increased salivation for 2 hours.
	219	31st May '35	7th June '35	Untreated . .	Showed parasites for 5 days after appearance.	...
	1094	31st May '35	1st June '35 to 5th June '35.	Do. .	...	'Carrier' animal parasites persisted throughout the period of observation.
Passage XVII.	571	7th June '35	11th June '35 Δ *B. big.*	11th June '35. 10 c.c. intravenously.	...	Died soon after the injection of 'Akiron R.' poisoning.
	58	7th June '35	7th June '35	Untreated . .	...	Parasites persisted throughout the period of observation.
	55	7th June '35	10th June '35	Do. .	...	Do.
Passage XVIII.	639	13th June '35	16th June '35 Δ *B. big.*	16th June '35 5 c.c. sub-cutaneously.	Negative following day till the period of observation.	Thermal reaction lasting 48 hours; local swelling.
	500	13th June '35	16th June '35 Δ *B. big.*	16th June '35 5 c.c. intramuscularly.	Parasites not seen again as above.	...
	370	13th June '35	16th June '35	Untreated Control	...	Parasites persisted throughout the period of observation.

TABLE II-A—*contd.*

No. of animal		Date of infection	Date on which parasites appeared	Date of treatment and dose	Results of blood smears	Remarks
Passage XIX.	Hill bull 712	19th June '35	22nd June '35 $\underline{\Delta}$ *B. big.*	22nd June '35 5 c.c. sub-cutaneously.	Negative the following day till the period of observation.	Slight thermal and local reaction.
	341	19th June '35	22nd June '35 $\underline{\Delta}$ *B. big.*	22nd June '35 5 c.c. intramuscularly.	Parasites not seen again as above.	Thermal reaction lasting 48 hours. Slight lameness.
	707	19th June '35	22nd June '35	Untreated Control.	...	Parasites persisted throughout the period of observation.

$\underline{\Delta}$=Very rare. Δ=Rare.

TABLE II-B.

No. of animal		Date of infection	Date on which parasites appeared	Date of treatment and dose	Result of blood smears	Remarks
Passage XX.	Hill bull 715	28th June '35	1st July '35	4th July '35 ++*B. big.* 5 c.c. sub-cutaneously.	24 hours after treatment parasites appeared degenerated and decreased; at 48 hours smears negative till the period of observation.	Appreciable thermal reaction for 48 hours; local swelling.
	686	28th June '35	4th July '35	6th July '35 +*B. big*. 5 c.c. intramuscularly.	5 hours after treatment parasites appeared degenerated, smears negative at 24 hours and after.	Increased salivation noticed for about one hour after treatment.
	638	28th June '35	2nd July '35	Untreated Control	...	Parasites persisted.
Passage XXI.	730	4th July '35	5th July '35 to 8th July '35	Untreated . .	Δ *B. big* . .	'Carrier,' parasites did not increase.
	305	4th July '35	9th July '35	Do. .	Δ *B. big* . .	Parasites did not increase.
	669	4th July '35	5th July '35	Do. .	Control . .	Parasites persisted.
Passage XXII.	465	12th July '35	15th July '35	18th July '35 +++*B. big.* 5 c.c. sub-cutaneously.	24 hours after treatment a small number of degenerated parasites seen. Negative at 48 hours and thereafter.	Marked thermal reaction for 24 hours after treatment.
	41	12th July '35	15th July '35	18th July '35 ++*B. big.* 5 c.c. intramuscularly.	Do. .	Slight thermal reaction for 24 hours with inappetence.
	558	12th July '35	16th July '35	Untreated Control	...	Parasites persisted.

+=few. ++=frequent. +++=numerous.

TABLE II-B—*contd.*

No. of animal		Date of infection	Date on which parasites appeared	Date of treatment and dose	Results of blood smears	Remarks.
	Hill Bull					
Passage XXIII.	674	18th July '35	19th July '35	25th July '35 ++*B. big.* 5 c.c. intramuscularly.	24 hours after treatment a small number of degenerated parasites seen. Negative thereafter.	Marked thermal reaction for 48 hours after treatment.
	721	18th July '35	24th July '35	25th July '35 ++*B. big.* 5 c.c. sub-cutaneously.	Do.	Slight thermal reaction, increased salivation for about an hour, inappetence for 2 days.
	681	18th July '35	24th July '35	Untreated Control	...	Parasites persisted.
Passage XXIV.	28	25th July '35	29th July '35	31st July '35 ++*B. big.* 5 c.c. sub-cutaneously.	24 hours after treatment a small number of degenerated parasites seen, negative thereafter.	...
	726	25th July '35	29th July '35	31st July '35 ++*B. big.* 5 c.c. intramuscularly.	24 hours after treatment parasites appeared degenerated and decreased, 48 hours a very small number of degenerated parasites seen and negative thereafter.	Inappetence for 2 days.
	42	25th July '35	29th July '35	Untreated Control	...	Parasites persisted.
Passage XXV.	41	31st July '35	4th Aug. '35	6th Aug. '35 +++ *B. big.* 5 c.c. sub-cutaneously.	Negative at 24 hours after treatment till the period of observation.	Thermal reaction for 24 hours after treatment.
	709	31st July '35	3rd Aug. '35	6th Aug. '35 +++ *B. big.* 5 c.c. intramuscularly.	24 hours after treatment a small number of degenerated parasites seen, negative thereafter.	...
	10	31st July '35	2nd Aug. '35	Untreated Control	...	Parasites persisted.
Passage XXVI.	4	6th Aug. '35	9th Aug. '35	Untreated . .	...	Parasites did not increase.
	12	6th Aug. '35	6th Aug. '35 & 11th Aug. '35.	Do. .	...	Do.
	644	6th Aug. '35	11th Aug. '35	Do. .	...	Do.

+ = few. ++ = frequent. +++ = numerous.

In connection with the work of testing the curative effect of 'Akiron R.' upon *B. bigemina*, it would appear from the foregoing tables that in all thirty-three bulls were artificially infected in a series of eleven batches, each comprising three animals. Of these, sixteen animals were subjected to treatment and the remaining served as controls. The first animal handled was No. 571 (passage XVII, Table II-A.) which died as a result of intravenous injection of 10 c.c. 'Akiron R.' The second animal No. 246 (passage XVI) received the same dose of the drug sub-cutaneously on the day that parasites were first observed, but it exhibited a severe

reaction soon after the introduction of the drug, in the form of foaming at the mouth and shivering lasting for about two hours; inappetence, rise in the body temperature lasting forty-eight hours; and a painful local swelling which persisted for a week. The day following, however, the parasites were not detected in blood smears from Hill bull No. 246, while the animal was under observation. In view of the toxic effects of 10 c.c. of 'Akiron R', the dose was reduced to 5 c.c. in the remaining animals. Of the four treated animals shown in Table II-A, with 5 c.c. doses, two were injected sub-cutaneously and the other two intramuscularly, on the day the parasites were detected in their blood smears. These four animals also showed a mild reaction to the drug, but none of them revealed the presence of *B. bigemina* during a period of observation of ten days.

Being encouraged by this finding more extensive trials were undertaken as indicated in Table II-B. In this series, the multiplication of the parasites was allowed to progress to various degrees, before treatment was given. Ten bulls were treated in different stages of the disease, leaving eleven animals to serve as controls. It would appear that in all the treated cases the peripheral circulation was cleansed of the parasites within forty-eight hours and no evidence of any relapse was confirmed by daily microscopical examination of blood films for the following ten days. In the controls, however, the parasites persisted. The drug appeared to become absorbed quickly and in the case of Hill bull No. 686, from which smears were examined every hour, it was noticed that a large number of parasites were distorted and commencing to disintegrate five hours after the injection. Whether the drug exerts a destructive action on the chromatin or the cytoplasm or both could not be definitely determined; for, both these structures, as noticed in the stained smears, were affected, leaving an irregular mass of the remnant on the erythrocytes. The destroyed parasites were then found to be gradually disappearing. At first the nucleus appeared as a speck of pink colour surrounded by a distorted, shrunk and deeply coloured cytoplasm. Later, in place of the nucleus, a small vacuole appeared within a dark mass of cytoplasm. This could then be easily mistaken for an artefact.

The curative effect of 'Akiron R' for *B. bigemina* infection, as would appear from the foregoing experiments proved to be far superior to that of trypanblue, for, a complete sterilization of the peripheral circulation was obtained within a short period. It will be observed that in most of the treated cases the dose of 5 c.c. of the drug produced a certain amount of reaction, and on this account, it was considered that a smaller dose may possibly yield identical results with advantage. The subsequent series of experiments were, therefore, directed towards the determination of a minimum effective dose of 'Akiron R'. Four trials were carried out as tabulated below (Table III), to test the efficacy of 1 c.c. on an average body-weight of 100 kg. The microscopical examination of blood films of such cases, however, showed that the reproduction of the parasites progressed unabated, proving thereby that this was too small a dose to effect a cure,

TABLE III

Results of treatment of B. bigemina *with 'Akiron R' at 1 c.c. dose*

	No. of animal	Date of infection	Date of treatment and dose	Results	Remarks
Passage XXVII.	Hill bull 87	15th Aug.'35	20th Aug.'35 : + *B. big.* 1 c.c. intramuscularly.	Parasites persisted throughout the period of observation.	......
	65	15th Aug.'35	20th Aug.'35: + *B. big.* 1 c. c. subcutaneously.	Parasites seen for 48 hours after treatment.	......
Passage XXVIII.	704	20th Aug.'35	Untreated Control .	......	Parasites persisted throughout the period of observation.
	34	20th Aug.'35	......	......	Did not react.
	733	20th Aug.'35	26th Aug.'35 $\overline{+++}$ *B. big.* 1 c. c. subcutaneously.	Parasites persisted for 4 days after treatment.	......
Passage XXIX.	486	26th Aug.'35	Untreated . .	......	Parasites persisted throughout the period of observation.
	14	26th Aug.'35	Ditto .	......	Ditto
	546	26th Aug.'35	31st Aug.'35 : $\overline{+++}$ *B. big.* 1 c.c. subcutaneously.	Parasites persisted throughout the period of observation.	......

+ = few. $\overline{+++}$ = fairly numerous.

In further trials the dose was raised to 2 c.c. per 100 kg. where by a total of five animals were treated at the height of infection as indicated below in Table IV-A.

TABLE IV-A

Results of treatment of B. bigemina *with 'Akiron R' at 2 c.c. dose*

	No. of animal	Date of infection	Date of treatment and dose	Results	Remarks
Passage XXX.	Hill bull 58	2nd Sept.'35	9th Sept.'35 : +++ *B. big.* 2 c.c. intramuscularly.	Cured .	A few degenerated parasites seen up to 24 hours after treatment and negative thereafter.
	88	2nd Sept.'35	9th Sept.'35 : +++ *B. big.* 2 c.c. subcutaneously.	Do. .	Ditto
	645	2nd Sept.'35	Untreated Control .	...	Parasites persisted throughout the period of observation.
Passage XXXI.	46	9th Sept.'35	17th Sept.'35: $\overline{+++}$ *B. big.* 2 c.c. intramuscularly.	Cured .	Same as in 58.
	72	9th Sept.'35	17th Sept.'35: +++ *B. big.* 2 c.c. subcutaneously.	Do. .	A few degenerated parasites seen up to 24 hours after treatment and negative thereafter.
	73	9th Sept.'35	Untreated Control .	...	Parasites persisted.
Passage XXXII.	1	17th Sept.'35	24th Sept.'35: $\overline{+++}$ *B. big.* 2 c.c. intramuscularly.	Cured .	Same as in No. 58.
	447	17th Sept.'35	......	...	Did not react.
	118	17th Sept.'35	Untreated Control .	...	Parasites persisted.

$\overline{+++}$ = fairly numerous. +++ = numerous.

The results incorporated in Table IV-A indicate that a dose of 2 c.c. of 'Akiron R' was sufficient and safe for successfully combating infection with *B. bigemina* in bulls averaging 100 kg. in body-weight. Furthermore, a few trials with 1·5 c.c. were also carried out but of the eight artificially infected bulls treated with this dose one animal continued to show the parasites for seventy-two hours after treatment, while in the others the circulation was free of parasites within forty-eight hours. Finally more tests were executed to confirm the curative effect of 'Akiron R' with 2 c.c. as shown in Table IV-B.

TABLE IV-B

Results of treatment of B. bigemina *with 'Akiron R' at 2 c.c. dose*

No. of animal		Date of infection	Date of treatment and dose	Results	Remarks
Passage XXXVIII.	Hill bull 149	29th Octr.'35	4th Novr.'35 : $\overline{+++}$ *B. big.* 2 c.c. subcutaneously.	Cured	A few degenerated parasites seen up to 48 hours after treatment and negative thereafter.
	148	29th Octr.'35	Ditto	Do.	A few degenerated parasites seen up to 24 hours after treatment and negative thereafter.
	158	29th Octr.'35	Untreated Control	...	Parasites persisted throughout the period of observation.
Passage XXXIX.	122	5th Novr.'35	13th Novr.'35 : + *B. big.* 2 c.c. subcutaneously.	Cured	Negative at 24 hours after treatment and thereafter.
	240	5th Novr.'35	Ditto	Do.	Ditto.
	139	5th Novr.'35	Untreated Control.	...	Parasites persisted for 10 days after appearance.
Passage XL.	249	13th Novr.'35	21st Novr.'35: $\overline{++}$ *B. big* 2 c.c. subcutaneously.	Cured	A few degenerated parasites seen up to 24 hours after treatment and negative thereafter.
	318	13th Novr.'35	21st Novr.'35: + *B. big.* 2 c.c. subcutaneously.	Do.	Ditto.
	315	13th Novr.'35	Untreated Control	...	Parasites persisted throughout the period of observation.
Passage XLI.	293	21st Novr.'35	28th Novr.'35: $\overline{+++}$ *B. big.* 2 c.c. subcutaneously.	Cured	A few degenerated parasites seen up to 24 hours after treatment and negative thereafter.
	261	21st Novr.'35	28th Novr.'35: +++ *B. big.* 2 c.c. subcutaneously.	Do.	Ditto.
	312	21st Novr.'35	Untreated Control	...	Parasites persisted for 4 days after first appearance.
Passage XLII.	165	28th Novr.'35	6th Decr.'35 : ++ *B. big.* 2 c.c. subcutaneously.	Cured	A few degenerated parasites seen up to 24 hours after treatment and negative thereafter.
	291	28th Novr.'35	Ditto	Do.	Ditto.
	183	28th Novr.'35	Untreated Control	...	Parasites persisted throughout the period of observation.

+ = few. $\overline{++}$ = fairly frequent. ++ = frequent. $\overline{+++}$ = fairly numerous. +++ = numerous.

In this series a total of ten animals were treated at varying stages of the disease and in all cases *B. bigemina* disappeared from the peripheral circulation within forty-eight hours after the treatment with a single subcutaneous injection of 2 c.c. of 'Akiron R'. In none of the treated cases did a relapse occur during a post-treatment period of observation of ten days. It has since been used as routine treatment in cases of resuscitation of *B. bigemina* in rinderpest work as well as remedy for obtaining '*bigemina*-free' rinderpest bull virus. Over fifty animals have been treated under the former category with a single subcutaneous injection of 1 c.c. for 100 lb. body-weight yielding 100 per cent cures. Its effect in yielding parasite-free rinderpest bull virus was experimentally tested on five artificially infected animals. These bulls were injected with 5 c.c. 'Akiron R' on the day they were used as rinderpest virus producers and in no case did *B. bigemina* appear during the course of the disease (rinderpest). As a control, similar animals, nine in number, were used as virus producers without being treated previously with 'Akiron R' and in seven cases out of nine *B. bigemina* was resuscitated within three to seven days after infection with rinderpest. It may however, be pointed out that for obtaining 'parasite-free' rinderpest virus from 'carrier' bulls treated with 'Akiron R', the infective material should be free from *B. bigemina*.

As a prophylactic against *B. bigemina* infection, 'Akiron R' proved to be inert as observed in another series of experiment. Twenty bulls were used for the purpose in the following manner :—

Two bulls were injected with 5 c.c. 'Akiron R' subcutaneously and infected with *B. bigemina* 10 days later.	Both relapsed.
Two bulls were infected after the same process 8 days later	Ditto.
Two bulls were infected after the same process 6 days later	Ditto.
Four bulls were infected after the same process 4 days later	Three relapsed and one proved refractory.
Two bulls were infected after the same process 2 days later	Both relapsed.
Four bulls were infected after the same process 24 hours later.	Two relapsed, one proved refractory and one died of other causes.
Four bulls were injected with 5 c.c. 'Akiron R.' and *B. bigemina* blood simultaneously.	Two animals relapsed, one reacted on test later and one proved refractory on test.

Lastly, an attempt was made to gauge the duration of cure that 'Akiron R' is capable of imparting after treating artificially infected bulls. A batch of twelve healthy bulls was infected with the laboratory strain of *B. bigemina* and treated soon after the development of the parasites with a single subcutaneous dose of 5 c.c. each and housed in an out-kraal. After the treatment was completed these animals were allowed to mix with the others in the same shed and left to graze out in the fields. Clinical observations were recorded and blood smears were examined daily for a period of twenty-two days in four cases and seventy-five days in the remaining eight cases. During this period three animals showed rare *B. bigemina* only once on 21st day after the treatment while the rest were negative throughout. Whether in these three cases the reappearance of the parasites could be attributed to a relapse or whether the infection was superimposed through the agency of the specific vectors, of which there appeared to be no dearth in that area, could not be definitely determined in view of the fact that these animals were not kept under tick-proof conditions. Seven clean animals were subsequently used as rinderpest virus producers after a period of five to six months and in no case *B. bigemina* was found to resuscitate. From these results, it is evident that the curative effect of 'Akiron R' is sufficiently lasting. Two other animals from the same batch of twelve bulls were infected with *B. bigemina* five months later and both developed the infection. The drug 'Akiron R' has been put on the market under the name of 'Acaprin' and is sold in 1, 5 and 10 c.c. ampoules in a 5 per cent solution. Smythe [1936] has successfully used 'Acaprin' in the treatment of red-water in cattle due to *B. bovis* infection. Cernaianu and collaborators [1936] report to have cured twelve bovines in 1934 showing severe symptoms of *B. bigemina* infection, with 2-5 c.c. doses of 'Acaprin'. In 1935, they treated fourteen animals obtaining nine recoveries as a result of one injection of the drug. Professor Horlein [1936] of the Bayer laboratories summarizing the results so far obtained with this product states that—

> "It exerts a specific and energetic action in almost all the piroplasmosis of the domesticated animals and it possesses a wide sphere of therapeutic action ; it is possible to administer it by subcutaneous, intramuscular and intravenous injection and also per orally. It is a colourless substance and there is, therefore, no staining of the tissues".

Summary

1. In routine practice a large number of failures resulted in the control of *B. bigemina* in cattle during rinderpest work with trypanblue. Controlled experiments were undertaken to test the efficacy of larger doses of trypanblue in bulls artificially infected with the local strain of the red-water parasites, but it failed to produce sterilization of the blood.

2. A proprietary preparation 'Akiron R', manufactured by Messrs. Bayer & Co. was tried with very successful results. The maximum effective dose of the drug was experimentally determined to be 1 c.c. of a 5 per cent solution per 100-lb. body-weight.

3. 'Akiron R' was found to be entirely valueless as a prophylactic agent.

4. The curative effect of the drug proved to be sufficiently lasting.

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THE EFFECT OF HIGH PROTEIN FEEDING ON MILCH COWS

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AMONG the several factors which have been studied for increasing the milk-yield of cattle, considerable attention has been paid to the question of protein intake and its possible effect on the quality and quantity of the milk. Weiser [1928] observed that when the digestible protein feed of a cow was lowered from 1·534 kg. to 0·871 kg., there was no difference found in the milk-yield. Savage and Harrison [1931] supplemented their roughage ration with concentrate mixtures containing 16, 20 and 24 per cent of protein and found that the different levels of protein feeding were of equal efficiency for purposes of milk-secretion. Henke and Goo [1933] compared the milk production on rations containing 10·8 and 20·2 per cent of digestible crude protein and noticed no change. Horwood and collaborators [1933] have shown that a concentrate mixture of 9·3 per cent digestible protein content was as good a supplement as one containing 16·7 per cent in maintaining the milk-yield, health and body-weight of cows on pasture. Further work by the same authors [1936], where the above mixtures were fed at the rate of 1 lb. for every 5 lb. of milk, showed that there was an increase of 3·6 per cent in milk-yield with the higher protein intake. Working on four dairy cows, Stewart and Tocker [1936] fed rations with widely differing albuminoid ratios, 1 : 2·76 and 1 : 9·90, with no appreciable alteration in the milk-yield.

The feed as a factor in influencing the composition of milk has also been the subject of study in some publications. Brouwer [1931] has stated that while the fat and some fat-soluble substances of milk may be altered in quantity and nature by a change in the feed, the protein and calcium in milk are relatively independent of the composition of the feed. Perkins and co-workers [1932] have found that the plane of protein intake had little effect on the composition of milk, except as regards non-protein nitrogen which increased a little with increased intake. According to the findings of Stewart and Tocker [1936] a high protein ration decreased the solids-not-fat and lactose in milk and increased the total nitrogen and albumin nitrogen, while the fat or ash content remained unaltered. Studies by Turner [1936] of average data from different laboratories have shown that, generally, the protein and ash in milk vary directly with the fat-content while the lactose fraction does so inversely. High protein feeding levels, however, had very little influence on the composition of milk.

It will be seen from the above review that no definite conclusions can be drawn on the effect of varying the level of protein feeding on the milk-yield of cows. An experiment was, therefore, conducted on similar lines to find out the response of cows under Indian conditions to an increased protein intake. The results obtained are presented in this paper.

Experimental

Twelve selected animals were divided into two groups so that their average age, live-weight, stage of lactation and milk-yield were about equal. All the animals were fed according to the computed requirements for maintenance and milk production. The feed consisted, on the dry basis, of about 18 lbs. of roughage, one-third of it being as *jowar* silage and Guinea grass and the rest as *ragi* straw. For maintenance requirements alone this roughage had to be supplemented by 12 oz. of groundnut cake. The animals received, besides, 1 lb. of concentrate mixture for every 2 lb. of milk-yield. The difference in the levels of protein intake between the two groups was effected by increasing the protein content of one of these mixtures, without greatly altering its energy value. All the animals were kept under identical conditions, the feeding and milking being done twice a day.

The following table (Table I) shows the composition of the two concentrate mixtures which were fed during the entire experiment. The digestible nutrients have been calculated on the basis of the coefficients given by Henry and Morrison.

It will be seen that the mixtures (Table I) provided a production ration containing 20·7 per cent and 29·1 per cent of digestible crude protein for the normal and high protein groups respectively. It may also be mentioned that the maintenance ration yielded 0·5—0·6 lb. of digestible protein for the average animal under experiment. On this basis a regular feeding trial was conducted, a daily record of actual food consumption, milk-yield and live-weight being maintained for individual cows. The results obtained may be considered under two different heads.

Quantity of milk-yield

The data obtained have been reduced to average daily performances of the average cow of each of the two groups, over monthly periods. Such a presentation would only show the broad effects, if any, which the increased protein intake may have had on the quantity of milk produced. Table II gives the results obtained from the first experiment. Table III shows the figures of a second experiment, carried out two years later, on the same basis, but with a different set of cows. These tables also give the average daily food consumption, milk-yield and live-weight of each of the groups during the respective experiments.

TABLE I

Composition of concentrate mixtures

Concentrates	Normal protein group			High protein group		
	Weight in lb.	Digestible crude protein lb.	Starch equivalent lb.	Weight in lb.	Digestible crude protein lb.	Starch equivalent lb.
Groundnut cake	3	1·443	1·588	5	2·405	2·647
Wheat Bran.	4	0·474	1·688	3	0·355	1·266
Gram husk	2	..	0·974	1	..	0·487
Brewery grains	1	0·149	0·876	1	0·149	0·876
Total	10	2·066	5·126	10	2·909	5·276

TABLE II

Average daily food consumption, live-weight and milk-yield

Period	Normal protein group					High protein group				
	Rough-age in kg.	Concen-trate mixture in kg.	Total feed in kg.	Milk-yield in lb.	Live-weight in lb.	Rough-age in kg.	Concen-trate mixture in kg.	Total feed in kg.	Milk-yield in lb.	Live-weight in lb.
December . . .	6·370	5·262	11·632	23·23	743	6·464	5·056	11·520	22·23	757
January . . .	5·843	4·811	10·654	20·45	729	5·578	5·245	10·823	22·09	749
February . . .	5·342	4·232	9·574	17·73	728	4·525	4·927	9·452	20·66	751
March . . .	5·403	4·144	9·547	16·70	740	4·657	4·433	9·090	18·19	759
April . . .	5·248	3·545	8·793	14·62	735	4·757	3·929	8·686	16·25	759
May . . .	5 176	2·919	8·095	10·57	733	4·848	3·478	8·326	14·08	762
June . . .	5·445	2·498	7·943	8·60	745	5·193	2·975	8·168	11·17	755
July . . .	5·729	2·795	8·524	11·04	732	5·692	2·486	8·178	8·86	756
August . . .	5·523	3·257	8·779	11·82	720	5·394	2·486	7·880	8·90	734
September . . .	..	..	..	..	..	..	..	..	..	..
October . . .	4·822	4·291	9·113	17·05	684	5·212	3·168	8·380	11·33	726
November . . .	5·321	4·541	9·862	18·84	701	5·347	2·955	8·302	12·58	710
Average .	5·475	3·845	9·320	15·51	726	5·242	3·740	8·982	15·12	747

TABLE III

Average daily food consumption, live-weight and milk-yield

Periods	Normal protein group					High protein group				
	Rough-age in kg.	Con-centrate mixture in kg.	Total feed in kg.	Milk-yield in lb.	Live-weight in lb.	Rough-age in kg.	Con-centrate mixture in kg.	Total feed in kg.	Milk-yield in lb.	Live-weight in lb.
December	5·657	6·079	11·736	28·60	822	6·145	6·017	12·162	27·60	839
January	5·704	5·890	11·594	26·58	831	6·186	5·889	12·075	26·30	852
February	5·760	5·437	11·197	24·22	841	5·937	5·215	11·152	22·84	863
March	5·713	5·041	10·754	21·71	851	6·142	4·815	10·957	20·70	870
April	5·700	4·342	10·042	18·69	848	6·105	4·367	10·472	18·46	872
May	5·675	3·829	9·504	15·95	850	6·221	3·877	10·098	16·17	884
June	5·596	3·360	8·956	12·85	861	6·222	3·500	9·722	13·13	898
July	6·039	2·785	8·824	9·72	879	6·450	2·966	9·416	9·15	922
August	6·283	2·214	8·497	4·85	891	6·465	2·399	8·864	6·40	939
September	5·925	1·844	7·769	2·49	889	6·032	2·512	8·544	8·43	917
October	5·955	3·431	9·386	14·13	900	5·859	3·935	9·794	16·91	866
November	5·059	5·894	10·953	26·36	844	5·314	5·092	10·406	21·25	867
Average	5·756	4·179	9·934	17·18	859	6·090	4·215	10·305	17·28	882

The following conclusions can be drawn from the data presented in the above tables. The extra protein feeding meant an increase of about 1½ lb. of oil-cake per day over the already rich concentrate ration of the normal group animals. It is interesting to note that this did not in any way affect the roughage consumption of the high protein group. The total food consumption per day remained roughly the same for both the groups. The increased intake of protein made no significant changes in the quantity of milk-yield. It is also apparent from their live-weight records that the extra protein fed was not utilized to produce an increase in the body-weight of animals. As the low protein group received a production ration containing about three times the protein secreted in the milk, these experiments do not yield any useful information regarding the efficiency of milk-protein production.

Quality of the milk produced

As these experiments were started mainly with the idea of studying the quantitative aspect of milk production, only a few samples of milk representing the two groups were analysed during their progress. The available data are given in Table IV.

TABLE IV

Composition of milk (per cent)

A = Normal protein group B = High protein group

Sample No.	Total solids		Protein		Fat		Sugar		Ash	
	A	B	A	B	A	B	A	B	A	B
1	13·02	12·37	2·90	2·87	4·5	4·1	4·9	4·4	0·695	0·744
2	12·70	12·26	2·90	3·02	4·5	4·2	4·5	4·5	0·679	0·737
3	12·62	12·21	2·94	3·04	4·5	4·1	4·8	4·6	0·681	0·742
4	12·65	12·23	2·96	3·03	4·4	4·1	4·6	4·3	0·689	0·750
5	12·49	12·43	3·09	3·26	4·3	4·5	4·6	4·4	0·703	0·750
6	12·89	12·00	2·98	2·97	4·6	4·0	5·1	4·4	0·688	0·753
7	14·73	14·25	3·38	3·54	5·6	5·1	5·1	5·0	0·677	0·733
Average	13·01	12·54	3·02	3·10	4·6	4·3	4·8	4·5	0·687	0·744

The last of the analyses given above refer to individual samples only as most of the animals had gone dry by that time. It may also be added that the figures in all cases represent the average of duplicate determinations.

Although definite conclusions may not be drawn from the above data, it is as well to state briefly the tendencies which this preliminary study reveals. It may be observed that the percentages of fat, sugar and total solids seem to be generally higher in the normal protein group. Particular attention might, however, be drawn to the ash-content of the two groups. The high protein group has consistently yielded milk of a higher ash-content.

In some cases the proteins have been further analysed and the casein and albumin content of the samples of milk are shown in Table V.

TABLE V

Composition of the proteins of milk (per cent)

A = Normal protein group B = High protein group

Sample No.	Total N		Casein N		Albumin N		Non-protein N	
	A	B	A	B	A	B	A	B
1	0·465	0·460	0·354	0·351	0·028	0·018	0·083	0·091
2	0·462	0·482	0·229	0·238	0·036	0·032	0·197	0·213
5	0·493	0·521	0·378	0·391	0·052	0·060	0·064	0·070
6	0·477	0·475	0·368	0·354	0·044	0·045	0·065	0·076
7	0·530	0·554	0·427	0·412	0·073	0·079	0·031	0·063

There appears to be a slight increase in the non-protein nitrogen fraction which may be attributed to the extra protein feeding.

Two main ingredients of the ash of milk, lime and phosphoric acid, have also been determined in all the above instances and are shown in Table VI.

TABLE VI

Lime and phosphoric acid in milk (per cent)

Sample No.	Ash		CaO		P_2O_5	
	A	B	A	B	A	B
1	0·695	0·744	0·155	0·159	0·159	0·155
2	0·679	0·737	0·153	0·167	0·200	0·204
5	0·703	0·750	0·163	0·173	0·200	0·202
6	0·688	0·753	0·153	0·163	0·190	0·181
7	0·677	0·733	0·159	0·171	0·204	0·213

It may be observed that the higher ash-content of the high protein group is consistently reflected in its lime-content also.

In conclusion, it may be recalled that the protein and lime in milk are generally held to be independent of the nature of the feed, though some evidence to the contrary has been put forward by Perkins and co-workers [1932] and Stewart and Tocker [1936] in regard to the nitrogen-content of milk. It is not possible, therefore, to evaluate the significance of the findings reported in this paper regarding the change in quality of the milk produced by cows under increased protein feeding. They have to be corroborated by more detailed studies covering a series of lactations with the same animals.

SUMMARY

An experiment has been made to study the effect of an increase in the level of protein feeding on the quality and quantity of milk-yield of cows.

Besides their requirements for maintenance, one group of six cows was fed a production ration containing 20·7 per cent of digestible crude protein, while that of the higher protein group, also consisting of six similar cows, contained 29·1 per cent of digestible crude protein.

No difference was observed between the two groups in food consumption, milk-yield or body-weight.

The milk from the high protein group showed a slight tendency to be richer in non-protein nitrogen, ash and lime contents.

The author is greatly indebted to Dr. F. J. Warth for his valuable guidance throughout as also to Dr. K. C. Sen for his great encouragement. Thanks are also due to Mr. Z. R. Kothavalla, Imperial Dairy Expert, Bangalore, for providing the animals under experiment and their feed.

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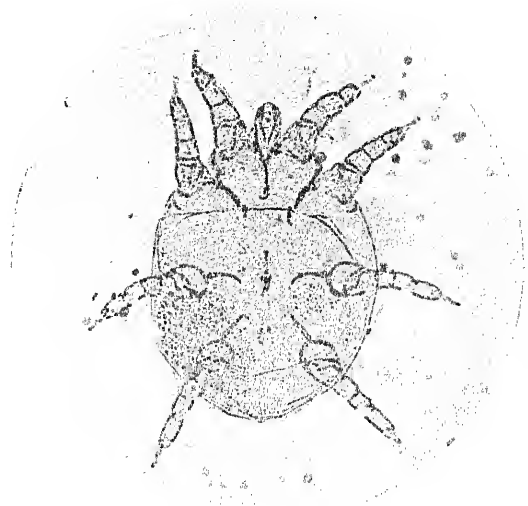

FIG. 1. *Cytoleichus nudus*. Female, ventral aspect.

FIG. 2. *Cytoleichus nudus*. Male, ventral aspect.

THE OCCURRENCE OF AIR-SAC MITE, *CYTOLEICHUS NUDUS* (VIZIOLI, 1870), IN FOWLS IN INDIA.

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(With Plate XXI)

CERTAIN mites, like the depluming mite, scaly-leg mite, feather mite, etc., attack the superficial parts of the fowl's body whereas certain fowl mites are deep-seated in the body like the air-sac mite and the connective tissue mite. The deep-seated mites are in reality degraded scab mites.

A perusal of text-books on Entomology shows that the air-sac mites are known to occur within the bones, lungs, bronchi, peritoneal cavity, etc. but in India, the authors have neither met with the occurrence of mites within the body of fowls except in the case under review nor have they noted any published record of its occurrence in India.

Pillers [1921] considered that this mite is parasitic upon birds and that the lesion resulting from its infestation rarely ends fatally. However, many writers incriminate it as a cause of inflammatory lesions in the lungs and intestines. Kaupp [1922] after studying a number of specimens considers that the air-sac disease caused by minute mites is fairly widespread in Colorado, North Carolina, New York and Pennysylvania. He records that the presence of a few parasites may not cause any noticeable symptom, but when they severely invade the bronchi in large numbers they cause local irritation, coughing, accumulation of mucus in the trachea and bronchi, partial asphyxiation, broncho-pneumonia, emaciation and death. Ward and Gallagher [1923] describe the air-sac mite as having been observed in the trachea, lungs, air-sacs, hollow bones and the peritoneum and exceptionally in the heart, liver and kidneys. Methods of transmission and their presence within the tissues cannot be readily explained. Small miliary tubercles containing the parasites may be seen scattered through the affected portion of the lung. Their exit from the body may occur through the trachea by expulsion

during coughing. Gwatkin and Glover [1931] have recorded their occurrence in the wind-pipe, lungs, air-sacs, hollow bones and the peritoneum, the air-sacs being the commonest site. They, however, consider that they are apparently harmless for they may sometime be found in large number in healthy birds.

The subject of report, from which the air-sac mites were recovered, was an ailing cock received from the Fowl Breeding Station of this Institute at Latoli, on 8th October, 1936, for observation with a view to eliminate the possibility of any contagious infection. The bird was kept in a cage and died on 13th October, 1936, prior to which thermal rise, inappetence and slight respiratory distress were noticed.

On post-mortem examination the entire peritoneal and thoracic cavities along with the viscera appeared as if sprinkled with sabulous deposits, which on microscopic examination were found to be accumulations of mites. They occurred in massive numbers and were seen to be actively motile on the surface. The lungs showed signs of consolidation, and the liver was studded with numerous pin-point to pin-head sized cream-coloured nodules. Some of the mites were found embedded superficially in the liver. The heart blood revealed no micro-organism on microscopic and cultural examinations. The death of the fowl resulted from pneumonia.

Pieces of the affected portions of the lungs and liver were sent to the Pathological section for a histopathological examination and report, which showed that the lungs had suffered from a severe type of pneumonia, the sections also exhibiting mites and their eggs along with rare groups of diphtheroids and Gram-positive bacilli. Liver showed marked congestion with foci of myelocytes (myelocytoma) some of which showed mitosis.

The mite on careful microscopic examination conformed in type to *Cytoleichus nudus* (Vizioli, 1870) given by the authors of various text-books on Entomology and Diseases of Poultry, referred to above. (Plate XXI, figs. 1 and 2). The parasite is ovoid in shape, yellowish white in colour and is just visible to the naked eye.

In view of the difficulty encountered in treating the affected fowls owing to the peculiar location of the parasite inside the body, it is considered that the condition is serious especially when infestation becomes heavy. It is advisable to destroy and cremate infested birds and thoroughly disinfect the premises. They should not be restocked for several months, during which period disinfection may be repeated.

Recently, a further consignment of dead or dying fowls has been received from the Fowl Breeding Station, Latoli, and on post-mortem examination heavy infestation with *Cytoleichus nudus* was found in each case. It appears that the flock and premises at Latoli are heavily infested with this mite.

Summary

The occurrence of mites within the air-sacs of fowls is recorded, for the first time in India. Although primarily parasitic in these sites, an extensive infestation of the organs had developed.

This mite is indistinguishable from *Cytoleichus nudus* (Vizioli, 1870), popularly known as the air-sac mite.

On account of the difficulty in treating the affected fowls it is considered to be a serious condition.

Acknowledgments

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A NOTE ON THE METHOD FOR THE DETERMINATION OF THE CALCIUM REQUIREMENT OF MAN

BY

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(Received for publication on the 3rd September 1937)

IN a very highly interesting paper on the determination of calcium requirement of man by Dr. I. Leitch, published in the *Nutrition Abstracts and Reviews* [January, 1937], the author has applied a new method to metabolism data, in which he divided 407 subjects into groups of negative and positive balances, based on the difference between intake and output of calcium. Out of 407 balances, 318 were negative and 89 were positive. Two linear relations, one with the negative and the other with the positive balance figures were obtained from which the maintenance requirement of calcium was estimated.

It appears that no justification has been given for dividing the 407 observations on normal individuals into two groupings except on balance basis which is the subject of investigation of the present paper. Assuming then the data to be normal, it is desirable to pool all the data together, neglecting the inevitable chance fluctuations from individual to individual and obtain one straight line (*i.e.* one relation) and estimate the maintenance requirement. In determining the protein and other nutrients requirements of Bengal bullocks, I have found this procedure to be very satisfactory.* For the application of the above method, the data of Table I, page 555 (*Nut. Abs. and Reviews.*, Jan. 1927) may be converted as follows :—

TABLE A

Calcium intake and output in normal women

Intake group	Positive and negative balances		
	No. of balances	Average intake in gram	Average output in gram
0·05—0·10 . .	16	0·080	0·220
0·10—0·15 . .	25	0·134	0·256
0·15—0·20 . .	11	0·176	0·246

* Unpublished work.

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TABLE A—*contd.*

Intake group	Positive and negative balances		
	No. of balances	Average intake in gram	Average output in gram
0·20—0·25 . .	33	0·225	0·346
0·25—0·30 . .	96	0·272	0·350
0·30—0·35 . .	77	0·316	0·390
0·35—0·40 . .	34	0·366	0·477
0·40—0·45 . .	28	0·438	0·489
0·45—0·50 . .	20	0·478	0·518
0·50—0·55 . .	43	0·518	0·534
0·55—0·60 . .	16	0·566	0·572
0·60—0·65 . .	1	0·630	0·680
0·65—0·70 . .	6	0·663	0·578
0·70—0·75 . .	1	0·742	0·547
Total . .	407	..	..

By the application of the method of least square, we obtain a relation :

$$Y=0{\cdot}640\ X+0{\cdot}187$$

Where Y represents the average output in gram, and X the average intake in gram. The high correlation coefficient ($r=0{\cdot}938$) indicates that the relation obtained between average output and average intake is in close agreement and that there is very little variation from individual to individual.

From the above relation it is easy to obtain the true maintenance requirement, by putting $Y=X=0{\cdot}52$ gram.

It may be added that Dr. Leitch's value is 0·55 gram.

REFERENCE

Nutrition Abstracts and Reviews 1936-37, **6**, 553-578.

SOME OBSERVATIONS ON THE INCIDENCE AND INHERITANCE OF IMPERFORATE ANUS IN GANJAM CALVES

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Introduction

The importance of the existence of lethal and sub-lethal factors in the economy of live-stock breeding deserves to be widely recognised. Numerous hereditary defects as a result of the above factors have been recorded among live-stock. The incidence of such lethal factors results in the death of the individual sometime between conception and a few days after birth.

Crew [1924] has given a detailed description of the condition (*Achondroplasia congenita*) or what is popularly known as the " Bulldog " calf of the Dexter breed of cattle which results in the birth of about twenty-five per cent of calves in a dead condition and with a monstrous appearance, their heads, limbs and vertebral columns being much foreshortened. Similar lethal factors operating in certain strains of the Scandinavian Telemark breed have been studied by Wriedt and Mohr [1925-26].

A lethal factor giving the calf the appearance of being devoid of skin and hair particularly on the muzzle and feet has been discovered by Hadley and Warwick [1927]. A lethal factor causing the large intestine to be completely cut off in the region of pelvic fixture of certain foals in Japan (*Atresia coli*) has been described by Yamane [1925 and 1927].

Various other hereditary defects, lethal or sub-lethal in character have been recorded by a host of workers in live-stock breeding—Loje [1930] in Red Danish cattle ; Roberts [1926 and 1929] in Welsh mountain sheep ; Mohr [1929] in pigs ; Kislovsky [1928] in rabbits, etc.

Imperforate Anus

A review of the literature on the numerous abnormalities in cattle reveals that the incidence of imperforate anus is one of rare occurrence. Kinzelbach

[1932] has recorded its incidence in pigs of the "Swabian Halle breed". Although there are isolated records of this abnormality in other places its occurrence *in extenso*, has been found only in the Ganjam Tract which now forms a part of the new Orissa Province.

In the administration reports of the Civil Veterinary Department, Madras, mention is made of the prevalence of this abnormality in the Ganjam District and of the operations carried out. In the following paragraphs, a record is made of the observations of the author which came to his notice in 1936-37 when he was in charge of the Veterinary Hospital at Berhampur (Ganjam District).

Observations

In the year 1936-37, 105 cases of imperforate anus were recorded. Of these, thirty-three were first calvings, twenty-one second, twenty-seven third, and twenty-four fourth calvings. The enquiry further elicited that in the case of cows which calved second, third or the fourth time, the preceding calves were normal. Thus it is obvious that most of the calves are produced in a normal condition and therefore the character of 'perforate anus' appears to be a dominant one.

The ryots of this tract, in cases of this abnormality, normally pierce the anus with a pointed red-hot iron and artificially create a faecal outlet. When veterinary aid is available, a surgical operation is performed. Most of the treated cases have been reported not to thrive well, but lead a lingering existence often resulting in early death. There is no authentic record to show that operated calves survive long enough to reach maturity. The possibility of utilising the recessive character 'imperforate anus' in crossing experiments may, therefore, be ruled out.

Accurate progeny records would be of great use in analysing the mode of inheritance of this interesting and widely prevalent character. It seems to the writer that a fair percentage of the cattle population of the tract may be tainted in this respect. Of the 105 cases, seventy-two were bull calves and thirty-three heifer calves. The higher incidence of this abnormality in bull calves is noteworthy and whether this is merely a chance or a phenomenon of normal occurrence remains to be ascertained.

Certain abnormalities such as congenital scrotal hernia, a small extra appendage of the skin just above or below the anal region, absence of prepuce in bull calves and hermaphroditism are found to be associated with some of the cases having imperforate anus.

Many of the breeding bulls and some of the cows in the tract have also been noted to possess the extra appendage of the skin in any part of the vertebral region; but the writer has not observed this near about the anal region as is found in some of the calves having imperforate anus.

It cannot be definitely stated whether the progeny of cattle having extra appendage of the skin in the vertebral region always exhibit greater incidence of imperforate anus and this is one of the questions, which requires to be pursued.

The fact seems to be that in the opinion of the ryots, the extra appendage of the skin present in a bull enhances the breeding bull appearance of the animal and the possibility of a lurking danger in perpetuating this abnormality (imperforate anus) does not appear to be realised.

Conclusions

It is obvious that the ultimate remedy lies in the elimination of this character from the Ganjam stock and the study of inheritance of this character is a necessary preliminary.

Acknowledgment

The observations made and recorded in this note are due to the incentive derived from the lectures of Professor William C. Miller, M.R.C.V.S., F.R.S.E., at the Institute of Animal Genetics, Edinburgh University, while working under him and the author's thanks are due to him.

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SELECTED ARTICLES

THE CONTROL OF ANIMAL DISEASES IN RELATION TO OVERSTOCKING AND SOIL EROSION

BY

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(Reprinted from the ***Empire Journal of Experimental Agriculture***, Vol. V, No. 18, April, 1937, 143-154)

SOIL erosion has been defined as 'the corrosive action of natural forces on the surface of the earth', and a certain amount of erosion, which in most places is more than balanced by rock decay, is a normal process. To-day, however, the term generally implies an accelerated loss of soil so far above the normal that it constitutes a very serious loss of the natural wealth of many countries. This accelerated loss is almost entirely due to disturbance by man or his domestic animals of the vegetative cover which alone can prevent the shifting of soil by rain and wind.

Throughout the world generally it is probable that injudicious cultivation is by far the most important source of soil erosion, and especially wherever the rainfall is both high and well distributed. Thus Eden [1] in his small monograph on soil erosion gave very little space to damage by stock, and the committee on soil erosion in Ceylon [2] did not mention stock at all.

It is in countries where the rainfall is low, or sufficiently seasonal to permit an annual dry season of several consecutive months, that damage by domestic animals may equal or exceed that done by the cultivator. Thus on page 12 of the Final Report of the Drought Investigation Commission of the Union of South Africa [3] it is stated that 'Deterioration in the vegetal covering of the drier parts of the Union has been brought about mainly through the practices of kraaling, herding, and overstocking, together with an insufficient number of drinking places, and overgrazing'; and on page 15, 'Soil erosion is caused, mainly, by deterioration of the vegetal cover brought about by incorrect veld management.' Also Bennett and Chapline [4] lay the blame for the appalling soil losses which are occurring in the United States of America almost equally on the shoulders of the cultivator and the stockman. We are not concerned, however, with apportioning blame; our present purpose is to emphasize the fact that overstocking is an important cause of soil erosion, and that wherever it exists it calls for serious consideration and scientific intervention. Even in those areas where there is as yet no apparent overstocking, the possibility of its occurrence should be borne in mind, and preventive measures be instituted when necessary.

What is overstocking? A definition which I have given elsewhere [5] is that 'Overstocking is the maintenance of animals on a piece of land to the detriment of its carrying capacity'. In the same article, I pointed out that such carrying capacity could be reduced without loss of soil or even of soil fertility, and that, therefore, soil erosion is not an inevitable sequel to overstocking. Nevertheless it is a common sequel, and the one which usually makes the solution of each overstocking problem an urgent necessity. In every way overstocking is altogether undesirable from the European point of view; an evil which, wherever found, should be removed if possible. The significance of the qualification 'European point of view' will be apparent later.

Since an avowed aim of every veterinary department is the control of animal diseases, particularly epizootic diseases liable to cause widespread and heavy mortality, it would appear that any progress towards the achievement of this aim must tend to increase stock populations, and may lead to overstocking with consequent overgrazing, or to an aggravation of these evils where they exist already. This view of disease-control is taken by many, and a common statement in local reports on soil erosion is to the effect that successful veterinary measures feed the roots of this evil. For example, in the Report of the Native Economic Commission, Union of South Africa [6], on page 14, we read: 'Measures for combating animal diseases have largely increased the number of cattle. Normally this should have increased the capacity of the country for carrying population, but the Natives' non-economic cattle outlook caused it to have the opposite effect'. And the Report of the Kenya Land Commission [7], on page 494, states: 'At the beginning of this century, in the country now known as Kenya Colony, the cattle had been suffering for the previous twenty years from a series of disastrous epidemics; in numbers they were comparatively few, and the grazing was more than ample to supply their needs. With the introduction of British Administration, veterinary measures for the control of these diseases were introduced, and by 1920 the cattle population had increased to an estimated total of 3,000,000. Up to that year signs of overstocking and consequent deterioration of land and cattle were hardly noticeable'.

Statements such as these naturally cause a government to doubt whether money allocated to its veterinary department is being spent in the country's best interest, and hesitancy in supporting every activity of their veterinary departments is apparent at the headquarters of many colonial governments of the present day. It is necessary, therefore, that each veterinary department examine its activities in the light of such criticisms, and ask itself if its policy of disease-control is compatible with a wider policy of rational stocking and soil preservation. Of such supreme importance to every country is its soil, that it is difficult to think of any economic desideratum which might justifiably be purchased by the sacrifice of much of this soil. Certainly the desideratum of disease-control should not be so purchased.

No one, of course, condemns the acquisition of knowledge which alone makes disease-control possible ; what is often deplored is the supposedly injudicious application of this knowledge. This critical attitude of mind is rational, and the challenge to veterinary departments is a fair one which must be met.

Having made this general concession, I wish now to say that I believe the challenge is one which can be met by every veterinary department, though naturally I am not in a position to speak for any other country than Tanganyika Territory. On behalf of the veterinary department of that country I readily accept the challenge, and as conditions there are not unique, it is probable that conclusions of wide application may be drawn from a careful examination of the facts relating to the one country alone.

Overstocking is prevalent in Tanganyika Territory. The area that is stocked to saturation is about 40,000 square miles, and probably 25,000 of these are overstocked, including what was some of the best land of the Lake, Central, Northern and Western Provinces. The subject has been given much attention and efforts to deal with the problem are being made. It may be said, though, that these efforts amount to no more than a beginning of what requires to be done if the evil is to be removed. In this connexion the recent annual reports of the Department of Veterinary Science and Animal Husbandry should be consulted, particularly the one for 1931, in which Staples deals at length with the problem as it occurs in the important Usukuma district of the Lake Province [8].

As more than 95 per cent of the stock of Tanganyika Territory is owned by natives, the problem of overstocking in this country may be considered as though it concerned native-owned stock alone.

It is important to note that overstocking is not a new thing in Tanganyika Territory. It is probably as old as animal husbandry itself; certainly it was observed by Stanley when he passed through Usukuma in September 1889. In his *In Darkest Africa* [9] he wrote : ' From our camp we could see the ancient bed of the Lake spreading out for a distance of many miles. Every half-mile or so there was a large cluster of hamlets, each separated from the other by hedges of milk weed. The plain separating these clusters was common pasture ground, and had been cropped by hungry herds as low as stone moss '. On p. 402 he wrote : ' The grass was so short that the cattle were feeding upon the roots to obtain subsistence '.

To some people these paragraphs indicate that overstocking in Usukuma was as bad fifty years ago as it is now, and therefore, as things are not getting worse, there is no need for worry. That this assumption is not justified will be shown later : what does emerge from all the evidence at our disposal is the conclusion that overstocking on a smaller scale existed before the days of European intervention.

How large the evil is at present may be gauged by the statement given above that 25,000 square miles are overstocked. 'It has been calculated that this area is still capable of carrying satisfactorily upwards of two million cattle together with the same number of sheep and goats, and actually it is being asked at present to sustain half as many more. Considering the cattle alone, this means that some 1,200,000 cows and heifers drop 600,000 calves every year on to land of which the carrying capacity is diminishing so that these births must be balanced by at least the same number of deaths from slaughter, disease, and starvation. By the most generous estimate 150,000 is the maximum slaughtered or sold for slaughter, and we cannot avoid the conclusion that an average of at least 200,000 die each year directly or indirectly from starvation. And meanwhile much of the land is steadily or, in places, rapidly, deteriorating' [5].

The primary cause of overstocking is the ordinary desire for wealth acting in communities where wealth is reckoned principally in terms of stock. Subsidiary causes are indifference to the future, and ignorance as to the best ways of achieving the desired wealth. The result is the acquisition by each individual owner of as many animals as possible with little regard to their quality, less regard to their standard of nourishment, and no regard to the way they deplete the soil. Such a state of affairs in a European community would be reprehensible ; in an almost savage community it is not blameworthy but merely deplorable.

One should not deal with the subject of native-owned stock without bearing in mind that, at present, to many natives no form of wealth can replace stock. To quote from Seligman's *Races of Africa* [10] : 'It is impossible to exaggerate the importance of their cattle to the Masai and kindred tribes ; not only must their practical function be considered, but also their ceremonial value, indeed the prominence that grass has in ritual among these tribes is due to the fact that it is the food of their beloved animals.

'Among the Suk, and this may be true of the other tribes, cattle are so important that if an adjective stands by itself the noun it qualifies is always understood to be " cow ". Again, in Suk even the skin of an ox has a different word to the skin of any other animal, and the verb to drink, if the fluid be milk, is different from the word meaning to drink any other liquid, while an ordinary gourd has a name different from that of one used to collect milk '. Again, on p. 175, Seligman says : 'Among the Dinka there is a well defined initiation ceremony at which the father of the young man presents his son with a bull, and it is no exaggeration to say that the youth attaches himself so strongly to this animal that the process called by psychologists " identification " takes place ; he will pass hours singing to and playing with his bull, he will be known to his associates by the name of his bull, and the death of the latter is a true bereavement. It is not then surprising that cattle are not killed for meat except on ceremonial occasions, the diet of the Nilotes being mainly milk and grain '.

Although the almost religious esteem in which pastoral tribes hold their cattle is weakening under the influence of education, it is still very strong and is never scoffed at by wise administrators. Many of us who have the welfare of the native at heart consider that his desire to possess many stock, if only it could be modified by consideration for the future as well as the present, is excusable, even laudable; as the man who succeeds is then capable of supporting his family in comfort and of contributing towards the public revenue.

The value of stock to the agriculturalist is particularly noticeable when drought, or the threat of drought, occurs during the season of crop growth. At such times people who are entirely dependent on their corn, *e.g.*, those who live in thick tsetse-fly belts, have a very anxious time. On the other hand, it is precisely during these same periods that stock thrive exceedingly, and much compensation for the depreciation of crops is afforded by the evening visits to the cattle-kraal, as the animals come home replete after a warm, dry day amidst abundance of nutritious, if wilted, grass. For it should be noted that little more than a month's drought in the growing-season may destroy the whole of a grain crop and yet do no permanent injury to grassland—it may destroy or prevent the seeding of many annual grasses, but that is unimportant if the perennials survive, as through these the pasture is replenished by rain coming too late to resuscitate the fields of maize and millet.

This apparent digression was necessary as it is not uncommon for a European who sees the desolation effected by soil erosion to forget the very real value of native-owned stock, and to think of them only as a pest which is destroying the land. The right conception is that although it is desirable for natives to possess stock, since these are a continual source of nourishment and clothing, and are an economic stand by if the grain crops fail, yet it is deplorable that ignorance and indifference should be permitted to turn a laudable industry into an economic disaster. One form in which this ignorance is particularly manifest is the native's apparent inability to realize that his present methods not only make bad provision for the future but are opposed even to his present interests. It is useless to point out to him that greater wealth results from maintenance of a smaller number of animals in good condition all the year round than a larger number in a state of semi-starvation. The truth of this general statement is unaffected by the consideration that most grazing is communal and therefore the individual would not benefit by a restriction which was not applied to all; and just as true is the statement that the average native cannot grasp the fact that this overstocking is reducing the grazing year by year, so that the total weight of stock which can be carried by any piece of ground must also become less and less. He thus neglects both his own interests and those of posterity.

We can now get an idea of the task which confronts any veterinary department when it is appointed to improve the stock industry of a country containing many native cattle. On the one hand there must be recognition of the fact that

domestic animals should be among a country's most valuable assets, and that every native who wishes to possess stock is entitled to attempt to do so, also that the special importance which pastoral tribes attach to cattle must be respected ; on the other hand, there is the realization that the natives' present methods of asserting their rights and privileges are prejudicial to general welfare.

A first task of such a department should be a survey of the distribution of stock. When this was done in Tanganyika Territory, a very unsatisfactory state of affairs was revealed.

No less than two-thirds of the Territory were found to be tsetse-infested and therefore free from cattle, and carrying only a sparse population of smaller stock. Two-ninths, although tsetse-free, carried a sparse animal population insufficient for the ordinary needs of the natives of the same areas ; and the remaining one-ninth was stocked to saturation.

The next step was to explain this distribution. The reason why the large tsetse-infested area is nearly free from stock is obvious but the explanation of the distribution of domestic animals on the fly-free areas was obtained only after some years of study, and is briefly as follows :

The two-ninths of understocked country are for the most part high-lands with granitic soils and a rainfall exceeding 30 in. Possibly the climax vegetation of such country is evergreen forest, but frequently recurrent fires now permit only a sub-climax of grassland or open deciduous savannah. The soils, too are leached and lateritic. The tall pyrophytic grasses which grow under these conditions furnish very great quantities of potential cattle-food so long as they are immature ; after maturity they lose almost all nutritive value, so that animals which are fat at the end of the rainy season lose condition rapidly throughout the cold dry months which follow. The same conditions of close grass-formation and comparatively cool moist climate are very favourable to the extra-host existence of ticks and parasitic worms, so that tick-borne diseases, notably East Coast Fever of cattle, and helminthiases, notably distomiasis of cattle and strongyloses of sheep and goats, are enzootic. Skin diseases, such as streptothricosis and follicular mange, affecting all classes of stock, are also prevalent. The net result is heavy disease infestation in a low nutritional environment ; a state of affairs leading inevitably to small flocks and herds composed of individuals which are heavily parasitized, frequently stunted, and of low fertility. There is usually a high mortality among young stock, and those which survive do so by virtue of acquired immunity to enzootic diseases imposed on an inherited high resistance to these diseases. Few imported animals can survive in such a locality unless they come from somewhat similar environment elsewhere. A somewhat surprising revelation is that although the cattle of these areas show such high resistance to local diseases, they offer little resistance to any unusual epizootic which may arise ; for example, an uncontrolled outbreak of rinderpest is usually disastrous in its consequences, and may completely exterminate a local cattle population.

The owners of the animals in these understocked highland areas are entirely unaware of the true causes why their stock do so badly, and they are extremely reluctant to accept any advice as to management which may be offered by a veterinary officer. Throughout the whole of this type of country there is an established equilibrium of low stock-concentrations which, when upset and readjusted, tends more frequently to lower than to higher concentrations. In other words, stock are dying out in some of these areas, and the already large proportion of the Territory which is without cattle tends to become yet larger.

Most of the one-ninth part of the Territory that is stocked to saturation carries a vegetation indicative of arid or sub-arid conditions. Some of it is naturally arid, and comprises open grass land, dry savannah, deciduous thicket, and cultivated land. In this kind of country surface-water is scarce, and stock populations are limited to the numbers which can find a bare livelihood within 15 or 16 miles of water—for to this great distance from water do stock graze in the dry season, drinking every second or even third day. Some of the land that is now sub-arid was not always thus; it has been reduced to this condition by the corrosive action of man's activities.

This condition of artificial aridity reveals an apparent biological anomaly which has been overlooked by most people, although it is of supreme importance in native animal husbandry.

It has been stated earlier that, for the European, overstocking is altogether undesirable: from the native's point of view, however, there is much to be said in favour of overstocking. Knowing nothing of the true cause of disease he yet takes advantage of the important fact that, under East African conditions, the denser a stock population is, the freer is it from disease, so that the healthiest flocks and herds are the ones living on the border line of starvation in arid and sub-arid districts.

I refer to this as apparently a biological anomaly, because the generally accepted view is that overcrowding favours disease. In animal ecology we think of populations of rodents or insects going in cycles; so long as the concentration of a species is low the average individual is healthy, but as numbers increase so disease becomes more rife until it culminates in an epizootic which brings the numbers down to a low level again. So with human beings; when considering problems of public health one accepts as almost axiomatic the view that overcrowding favours disease. Therefore, it is at first sight surprising that a concentration of domestic animals high enough to constitute overstocking should be healthy. And very healthy they often are. They almost starve during some months of every year and they rarely attain their full potential weight, but so well adapted are they to these conditions that, like the dwarf xerophytes which form their sustenance, they react rapidly to the better conditions of the months when food is more available, and during this time they put on flesh and attain breeding condition.

The explanation of the healthiness is to be found by studying the nature of the most devastating stock-diseases of these parts. They are due to parasites which pass part of their existence away from their mammalian host. Such parasites include piroplasms and rickettsia, which are transmitted by ticks, trypanosomes, transmitted by tsetse and nematodes. Of these transmitters, ticks generally leave their hosts for moulting and egg-laying, and tsetse are attached to their hosts only for brief minutes of feeding. Most parasitic nematodes, too, pass critical stages of their larval life on the ground. The conditions which are most unfavourable to these disease agents are furnished by bare land exposed to the hot sun; conditions which, in Africa, are always associated with aridity. This is explained why the parts of the Territory which are agriculturally the richest are frequently extremely unhealthy for stock, but may be made comparatively healthy by measures which favour erosion and bring about soil aridity even in the presence of good rainfall.

As the environment gets more and more arid from such a cause as overstocking, the animals appear to become smaller rather than fewer. This point is being investigated, but already there is evidence to indicate that the seasonal under-nourishment associated with overstocking tends, through survival of the fittest, to produce a dwarf race of full fertility, but low size-potential, rather than mere stunted individuals of low fertility and retaining a comparatively high size-potential.

This is probably an important point, as combined with the effect on disease prevalence, it means that this artificial aridity does not readily provide the seeds of its own remedy; the trend is towards a lower and lower equilibrium in which depleted soil is balanced by a more xerophytic flora and a more dwarf population of domestic animals. In this way we can reconcile the statement of modern economists that the great country of the Wasukuma lying south of Lake Victoria is being turned into desert, with the known fact that Stanley described a scene of obvious overstocking in these parts when he passed through them with Emin Pasha fifty years ago for there is little doubt that in the areas which were then overstocked the equilibrium between soil, vegetation, and domestic animals was higher than at present. The country *is* on its way to desert, as it was then, but only carefully recorded data, which are non-existent, could show clearly that it is a step nearer to desert now than it was then.

Naturally the native understands nothing of this. He does know, however, that he is continually faced with the alternative of exposing his cattle to the risks of starvation in dry areas, or of exposing them to the risks of such diseases as East Coast Fever in areas where grass is abundant. One might almost say that this is the chief problem in the lives of nomadic pastoral people, though with them the healthy regions are the naturally arid ones of low rainfall.

The real problem of overstocking in relation to soil erosion is furnished by the agriculturalists who are also stock-owners, such as the Wasukuma, to whom reference has been made. These settled in the areas of greater soil fertility and originally, no doubt, lost a good many stock from enzootic diseases. As the direct result of their erosive methods of agriculture and animal husbandry they rendered these areas less fertile but healthier for stock, which consequently increased to, and are now maintained at, saturation point.

This type of abused country is commonly known as *cultivation steppe*, and it exists in patches of varying size in every province. It is associated with a dense concentration of both natives and stock and with comparatively good water-supplies. Its measure is largely the measure of the badly eroded land of the Territory. Not only are conditions within areas of this type getting worse, but the areas themselves are spreading, like ulcers, wherever surface water permits their peripheries to be extended to meet the growing need of an increasing population driven outwards from centres of ruined fertility.

This analysis of stock-distribution seems to indicate clearly that unaided native husbandry tends to create the two extremes of under-and over-stocking. Prior to the European invasion of Tanganyika Territory there were large areas of overstocked land, and enormously larger areas of cattle-free country. Since the arrival of Europeans some formerly cattle-free country has become stocked, but there has been no decrease of overstocking ; rather has this evil considerably increased.

As the veterinary department is the branch of government which has most to do with the interests of the stock-owner, and since on our own finding these interests have in some respects deteriorated through increased over-stocking, it is natural that the department should be considered as in some measure responsible for the deterioration. This is a reasonable view which does not, however, justify the conclusion that disease-control is the main cause of accelerated overstocking.

The most important diseases of stock in Tanganyika Territory are rinderpest, contagious bovine pleuro-pneumonia, East Coast Fever, trypanosomiases, anthrax, contagious caprine pleuro-pneumonia, diseases due to worms, and skin diseases. There are about five million cattle and about the same number of sheep and goats, besides donkeys, pigs, dogs, poultry, etc. At the end of 1934 there were only fourteen veterinary officers in the department, including the Director and two research officers. Owing to furlough and sickness, there are never more than three quarters of this staff on duty at one time ; that is never more than ten veterinarians to deal with the diseases of ten million stock. They would be rather wonderful men if by their methods of disease-control they were able to cause overstocking.

It is true that the first *aim* of the veterinary department is the application of measures of disease-control to the extent that these are justified economically. I consider that an effort should be made to suppress completely both rinderpest and pleuro-pneumonia, and that staff, knowledge, and funds should be available to allow any other disease to be controlled locally at any time this becomes economically desirable.

But up to now the disease-control measures of the department have perforce been confined to a struggle to keep rinderpest and contagious bovine pleuro-pneumonia within bounds, and to deal with the other diseases when outbreaks assumed excessive local importance. The only significant way in which these attempts have influenced mortality returns is in connexion with rinderpest, so for the purpose of this article we can substitute the term rinderpest-control for the wider term of disease-control, and consider very briefly any effect which such control has had on overstocking.

Rinderpest is a contagious disease capable of infecting most species of ruminants and a few non-ruminants. In East Africa it affects particularly cattle and, among game animals, buffalo, eland, giraffe, kudu and wart-hog.

Within historic times the disease made its first appearance in Africa south of Egypt about 1889, when infected cattle were brought across the Red Sea from Asia. Once introduced it spread rapidly in the form of the greatest epizootic on record. As it swept through Somaliland (1889), Masailand (1890), Nyasaland (1893,) Rhodesia (1896), Transvaal (1896), and Cape Colony (1897), it almost exterminated the cattle and buffaloes in its path. Other game animals also suffered heavily.

So thorough was the destruction, that the epizootic soon died down for want of further large groups of animals to infect, and energetic measures by the governments of all the countries of South Africa succeeded in stamping out the embers. Thus by about 1905, the disease was completely suppressed in all countries south of what is now Tanganyika Territory (then German East Africa), nor, except for a brief incursion into Northern Rhodesia in 1919, has it been allowed to spread south again since. It is one of the main duties of the veterinary department of Tanganyika Territory so to control rinderpest within its bounderies that there is no danger of the disease spreading to its southern neighbours. Total suppression is aimed at but is unachievable with a small staff, and each annual report of the department records the number of outbreaks dealt with and the number of cattle inoculated. In 1934, there were 44 outbreaks and 130,000 cattle were inoculated.

It must be borne in mind that one attack of rinderpest confers almost lifelong immunity, and, for reasons which need not be discussed here, the form in which the disease occurs to-day in East Africa is much milder than the raging epizootic which worked such havoc in the final decade of last century. It is necessary only

to describe what occurs to-day in that part of Tanganyika Territory where the disease is hardly interfered with by the veterinary department. This part is the large and important cattle district of Musoma, lying east of Lake Victoria. Here the disease is always smouldering and sometimes flaring up into outbreaks of considerable size. In the herds where rinderpest is actually active, every susceptible beast, that is every beast which is not immune from a previous attack, may become infected. Some of these die, and some recover, and the disease passes on to other herds, to return again after a period of anything between one and ten years, usually after four or five years.

The most important aspect of this uncontrolled rinderpest is the mortality. Whereas the original epizootic on its way through Africa killed about 90 per cent of the cattle attacked, to-day in Musoma it kills a mere 5 to 40 per cent, depending on many factors. This means that when rinderpest is uncontrolled in a district like Musoma, every beast is likely to get it sooner or later, with an expected mortality of 20 per cent.

The normal composition of a Musoma herd of 100 head is 40 cows and 10 adult males, all of which may be immune to rinderpest, and 50 young stock of both sexes, which may be susceptible to the disease. If rinderpest comes along, these 50 become infected and 10 may die. But the 40 cows are capable of rearing 20 calves in a year, so that in the absence of mortality from any other cause the herd could increase even during the year of infection.

Musoma district adjoins Usukuma and resembles it in many ways. Many parts of Usukuma are stocked to saturation, which means that no further increase in numbers can take place—if the herd of 100 is not kept down to 100 by disease and slaughter, starvation will effect the adjustment. But whereas rinderpest takes toll of young vigorous animals, starvation destroys only the weak and aged. Both methods of culling are cruel and deplorable, but starvation does the job better.

Having demonstrated that rinderpest-control has no effect on overstocking, I must add that the main reasons why such control is attempted in most overstocked areas is not for the local effects, but to prevent the disease spreading from these areas, both to other countries and to the understocked parts of the same Territory. In these understocked parts the cattle are so debilitated by enzootic diseases (*vide supra*) that an outbreak of rinderpest may almost exterminate the susceptible animals. Thus a policy of *laissez-faire* with regard to rinderpest merely encourages the inevitable trend of native animal husbandry towards its two extremes of overstocking and complete absence of stock.

I do not say that disease-control never causes or increases overstocking. What I do say most emphatically is that in Tanganyika Territory it does little or nothing towards accentuating the bad conditions in the already overstocked areas. In this Territory, and doubtless in many others, the desideratum of disease-control is *not* being purchased with the indispensable soil.

Pax Europa is the main reason why overstocking has increased so much in East Africa during recent years. Before the settlement of Europeans the history of much of Africa was 'a tangled skein of secessions, wars, migrations, and exterminations' amid which even the strongest tribes had little chance to accumulate stock unmolested for long periods. To-day the weakest tribes tend their animals without fear. Coincident with the first decades of this era of peace was natural recovery of stock populations from the effects of the greatest epizootic of cattle in history.

Summary

Soil erosion is due to the disturbance by man or his domestic animals of the vegetative cover which alone can prevent the shifting of soil by rain and wind.

In many countries with a long annual dry season, overstocking is an important cause of erosion.

Since adequate disease-control tends to increase stock populations, many people have suggested that these measures favour overstocking.

These suggestions naturally raise a doubt concerning the economic value of some phases of veterinary activity in countries where overstocking is rife, and convey a challenge to all veterinary departments.

It would be presumptuous for an individual to reply to this general challenge, but the author uses it as a basis for a review of the situation in Tanganyika Territory where overstocking has been prevalent from time immemorial.

The primary cause of overstocking in Tanganyika Territory is the ordinary desire for wealth acting in communities where wealth is reckoned in terms of stock and where cattle are held in almost religious esteem. Subsidiary causes are ignorance and indifference to the future.

A brief review of the place which stock occupy in native agriculture is given, and the conclusion drawn that it is desirable for natives to possess stock, but deplorable that ignorance and indifference should be permitted to turn a laudable industry into an economic disaster.

With a view to finding some way of improving the stock industry in the face of many difficulties, the veterinary department of Tanganyika Territory made a survey of the distribution of stock. One most interesting result was the discovery that, generally speaking, the denser a stock population is, the freer it is from disease.

The explanation of this is given, and it is shown that the parts of the Territory which are agriculturally the richest are frequently extremely unhealthy for stock, but may be made comparatively healthy by measures which favour erosion and bring about artificial soil aridity. Unaided native husbandry tends to create the two extremes of under and over-stocking, so that the latter may be considered an essential feature of unaided native husbandry.

In Tanganyika Territory the only form of effective disease-control which can be carried out by a small veterinary staff is rinderpest-control, and the evidence is clear that this does little or nothing towards accentuating the bad conditions of the overstocked areas, since mortality from uncontrolled rinderpest in these areas is less than the usual average wastage from starvation.

In conclusion it is pointed out that the reason for the increase of overstocking in Tanganyika Territory during recent years is *pax Europa*, especially when this had effect at the time when native herds were recovering naturally from losses sustained during the greatest epizootic of cattle in history.

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PARASITE REACTIONS TO HOST MODIFICATIONS *

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IT is customary for the president of this society to make his address a discussion of some problem in which he is particularly interested. Continuing this custom, I wish to bring to your attention one method of attacking the great problem of host-parasite specificity. The term specificity as used by us does not refer to the specific identity of either host or parasite but to the specific or definite infection-relations existing between species of hosts and species of parasites. The use of the term may best be illustrated by several examples. We have found that in most cases each species of *Giardia* flagellate inhabits only one species of host and does not ordinarily live in other species of hosts in a state of nature. This we refer to as a rigid host-parasite specificity. On the other hand, the large ciliate, *Balantidium coli*, lives as a normal parasite in three species of hosts, namely, chimpanzees, pigs and man. A condition of this sort we consider to represent a loose host-parasite specificity in which a species of parasite lives in nature in several species of host. Or these same relationships may be described from the standpoint of the host as follows: almost every species of host is inhabited by its own peculiar species of *Giardia*; whereas three species of hosts, pigs, chimpanzees and man, are all inhabited by the same species of *Balantidium*.

Why one species of host is in general inhabited by a group of parasites that do not occur in other species of hosts, or why one species of parasite lives in one or only a few species of hosts instead of in others that seem equally satisfactory to us, are very intriguing problems. One method of determining the factors involved is to modify hosts by subjecting them to various conditions and to note the reactions of their natural parasites or of foreign parasites introduced into them. During the past fifteen years my colleagues, students and myself have carried on various investigations that throw light on these problems. Our investigations have included the use of modifications of a purely laboratory type as well as alterations that might more or less easily occur in nature. The latter are of particular interest because we wish especially to know what factors account for the host-parasite specificity observed under natural conditions.

*Address of the Retiring President, American Society of Parasitologists, December 29, 1936, Atlantic City, New Jersey. Those who took part in the investigations on which this discussion is based are as follows: Justin Andrews, G. H. Boyd, L. R. Cleveland, M. S. MacDougall, Lydia Eskridge, Arnoldo Gabaldon, Robert Hegner, E. C. Nelson, H. L. Ratcliffe, Eugene Schumaker, K. S. Shah, L. G. Taliaferro, and W. H. Taliaferro.

Most of the protozoa of higher animals may conveniently be separated into two groups, those that live in the digestive tract and those that live in the blood stream. The intestinal protozoa are most easily influenced by modifying the diet of the host and hence we have devoted a large part of our effort to this phase of the problem. Blood-inhabiting protozoa may also be influenced by changes in the diet of the host, since the blood carries the products of digestion to the tissues. Temperature, humidity, and muscular activity also may alter the condition of the host sufficiently to bring about visible reactions of the parasites. Drugs and sera of various sorts have been employed to change the course of infections, to eliminate parasites from the host or to vary the medium within the intestine or blood stream, but these agents could not conceivably operate in nature and our results along these lines are therefore omitted here.

With these few remarks as an introduction, I will now proceed to present a running account of our studies.

Our attention was first attracted to this subject while studying the intestinal protozoa of amphibians. Ciliates of the genus *Opalina* are commonly present in the intestine of tadpoles of various species of frogs. They persist in the adults of certain species of frogs but disappear in the green frog when the tadpoles of this species undergo metamorphosis and do not ordinarily occur in the adult green frog. Metcalfe suggested that perhaps the change from the vegetable food of the tadpole to the animal food of the adult might account for the loss of the opalinids. This explanation is not satisfactory since other species of frogs that retain their opalinids after metamorphosis also live on animal food as adults. So far as I know no one has yet explained why the adult green frog differs from other species of frogs with respect to the opalinid ciliates.

For several reasons the rat is more favourable than the frog for the experimental study of intestinal protozoa. We therefore transferred our attention to the rat and soon found that it was possible to modify this animal so as to influence profoundly its protozoan parasites. We noted first that certain rats that had been used by Dr. E. V. McCollum for dietary studies were free from trichomonad flagellates which are usually very abundant in the caecum. Inquiry revealed the fact that these rats had been fed throughout their lives on a diet high in animal protein. This led to experiments which gave what appeared to be conclusive results, since trichomonads disappeared from, or became much less numerous in, rats that were fed on such a diet instead of what we call a normal diet consisting largely of carbohydrates.

Then it occurred to us that the intestinal protozoa we were supplying our students for laboratory study came principally from herbivorous animals, and a review of the literature soon revealed the fact that very few intestinal protozoa have been recorded from carnivorous animals. The evidence thus seemed quite

convincing that for some reason a diet high in animal protein brings about a condition in the intestine that is unfavourable for the growth and reproduction of intestinal protozoa. This appeared to be true particularly of trichomonad flagellates which we studied more thoroughly than we did any other type.

The animal proteins that we used in our early experiments were contained in beefsteak. We next undertook to determine whether other animal proteins would have a similar effect. Rats were fed on diets containing 60 per cent of protein. The protein fed to one group of rats was derived from beefsteak, to a second from beef liver and to a third from casein. The results were quite striking. After 20 days rats fed only 20 per cent protein contained on the average about 10,000 trichomonads per cubic millimeter of caecal material, whereas rats fed on the beefsteak diet gave a count of only 1,000, those fed on the beef liver diet contained 28,000, but those fed on the casein diet only 260. It seemed quite evident from these results that different types of protein brought about conditions within the intestine of the host sufficiently different to affect drastically the welfare of the trichomonads.

Just recently we had an opportunity to try out a diet high in vegetable protein. Groups of infected rats were fed on a normal diet, on a diet high in animal protein and on a diet high in vegetable protein, respectively. The animal protein diet contained 70 per cent of casein and the vegetable protein diet contained 77 per cent of wheat gluten. The average number of trichomonads per cubic millimeter of caecal contents was 8,270 in rats fed on a normal diet, 200 in rats fed on animal protein and 1,137 in rats fed on vegetable protein. Apparently the caecal contents are adversely affected by the vegetable protein but not as unfavourably as by the animal protein.

Two factors occurred to us that might account for the situation revealed by these experiments: first, changes in the hydrogen-ion concentration of the intestinal contents, and second, changes in the relative number of different types of bacteria. A preliminary examination of control and experimental animals indicated that no differences in hydrogen-ion concentration were present that seemed great enough to account for the results noted. Later a more careful study was made. The hydrogen-ion concentration of the caecal contents of rats fed on a normal diet was on the average about 6·78, of those fed on a diet of beefsteak about 6·82, of those fed on a diet of beef liver about 6·42, and of those fed on a diet of casein about 7·12. Considering the wide range in the hydrogen-ion concentration of culture media in which trichomonads live successfully, it appears probable that this condition played a relatively minor rôle in the environmental factors responsible for the growth and multiplication of these flagellates.

Various investigators have demonstrated that profound changes occur in the bacterial flora of the rat's intestine when its diet is changed from one consisting

largely of carbohydrates to one high in animal protein. We were of the opinion from the very beginning that the effects on the trichomonads were due either to changes in the types of bacteria resulting from the ingestion of large amounts of protein or to changes in the products of bacterial activity or to both these factors. An extensive series of experiments seemed to indicate a definite relation between types of bacteria and numbers of trichomonads. The character of the results is indicated in the accompanying table. It will be noted that when rats were fed on either beef or casein the number of trichomonads decreased markedly in numbers, proteolytic anaerobes increased in numbers, fermentative anaerobes increased in numbers coli-aerogenes forms exhibited no definite trend and acidurics decreased in number.

	Av. no. trich.	Av. no. anaerobes		Av. no. aerobes	
		Proteo.	Ferment	Coli-Aerog.	Acidurics
20 per cent protein	10,570	2·7	54,000	3,047	537,000
Steak, 10 days	6,820	27·1	352,000	6,268	527,000
Steak, 20 days	880	37·0	361,000	8,050	188,000
20 per cent protein	16,850	112·4	55,000	3,552	1,070,000
Casein, 10 days	720	136·0	73,000	1,939	113,000
Casein, 20 days	260	532·0	73,000	1,174	40,000
20 per cent protein	10,151	125·0	45,000	620	810,000
Liver, 10 days	19,800	112·0	550,000	507,730	425,000
Liver, 20 days	28,000	0·4	2,440,000	1,350,000	96,000

On a liver diet the trichomonads increased in number, the proteolytic anaerobes decreased in number, the fermentative anaerobes increased in number the coli-aerogenes forms increased in number and the acidurics decreased in number. Since the proteolytic anaerobes were low in number on a normal diet, increased in number on the steak and casein diets and decreased in number on the liver diet, the conclusion seemed justified that these bacteria were responsible for rendering the intestinal contents unfavourable for the trichomonads. There was a decrease in the number of acidurics but this likewise occurred when the favourable liver diet was fed to the rats.

The data then available indicated that on any diet the number of trichomonads varied inversely with the number of proteolytic anaerobes present.

Sometimes a condition that is unfavourable for one species of protozoon may not have any obvious effect on other species of the same genus. This led us to test the effects of a high protein diet on the human trichomonad, *T. hominis*, in rats. Our discovery that rat trichomonads can easily be eliminated by carbarsone treatment gave us trichomonad-free rats to work with. These were infected orally with *T. hominis* grown in culture. Whe n fed

on a diet high in animal protein 5 of 10 rats became trichomonad-free, and in 2 others the trichomonads were too few to detect in smears but appeared in cultures. Only one of the control rats fed on a normal diet lost its trichomonads during the experimental period of 36 days. These data indicate that when rats infected with *T. hominis* are fed on a diet high in animal protein, the intestinal contents become unfavourable for them just as they do for rat trichomonads.

Surveys that are undertaken to determine the incidence of *T. hominis* in human populations are usually unreliable. Ordinarily many infections are missed for reasons I need not discuss at present. Occasionally an infection is noted that is accompanied by a diarrhoeic condition. A meat diet has been suggested for the treatment of such cases and a number of instances have been brought to my attention in which the results were very satisfactory both the diarrhoeic condition and the flagellates disappearing within a very few days. This suggests that a diet high in protein renders the intestinal contents of man unfavourable for *T. hominis* just as it does in rats.

I wish now to direct your attention to another method of attacking this problem using another species of intestinal protozoon. For the most part investigators have devoted their time and efforts to attempts to eradicate parasitic organisms, and comparatively few have endeavoured *to improve the environment in the host in favour of the parasite.* No one knows what factors prevent a certain species of parasite from living in a given species of host, but it might be possible to modify the host in some very simple way that might even occur in nature so as to render it susceptible to infection. We have tried to modify rat hosts so that they can be infected with balantidial ciliates to which they are ordinarily resistant in nature. Our procedure was based on the results obtained in our trichomonad studies. We fed rats on diets either high in carbohydrates or high in proteins. Balantidia obtained directly from the intestine of pigs, or grown in culture or taken from experimentally infected rats were inoculated into rats either intragastrically or intracecally. Eighteen rats fed on a diet consisting of 70 per cent casein failed to become infected. Balantidia were later eliminated within 8 days from 12 rats that had been experimentally infected, by feeding them on a diet high in casein. Of 49 rats fed on a diet containing 60 per cent carbohydrates and 15 per cent casein, 47 bacame positive and retained the infection for from 1 to 33 days. When the carbohydrates were increased to 85 per cent and the casein reduced to 5 per cent, 27 of 52 rats became infected and remained infected for from 3 to 44 days. A diet of 93·5 per cent carbohydrates, however, proved to be the most effective, since 89 of 101 rats became infected when this ration was used and the balantidia were retained up to 154 days. Furthermore, when rats infected on a 60 per cent or 85 per cent carbohydrate diet were changed to a 93·5 per cent carbohydrate diet the number of balantidia present increased markedly within from 5 to 8 days.

Another method of testing the influence of carbohydrates on balantidia was to inject starch in either gelatinized or granular form into the caecum of infected rats. In every case the number of balantidia increased greatly within a short time, whereas no increase in numbers occurred in the control animals. During these experiments both division and conjugation were noted among the balantidia, but no cysts were observed. The conclusion reached was that rats might become infected in nature if they were to ingest balantidia and were to subsist on a diet containing from 60 to 93·5 per cent of carbohydrates. They could not transmit the infection to other rats, however, since infective cysts apparently are not formed within the intestine of the rat.

Confirmation of these experimental results was obtained when caecal material from 79 pigs was analyzed. It was found that heavy infections with balantidia were generally accompanied by a diet high in carbohydrates in the form of grain. Large quantities of undigested carbohydrates and an intestinal flora such as would develop on a high carbohydrate diet were found in these animals and absent from pigs that were either negative or exhibited light infections with balantidia. Heavy infections with balantidia were accompanied by larger numbers of aciduric organisms and lesser numbers of lactose fermenters and proteolytic anaerobes, and light infections were accompanied by a lesser number of acidurics and a greater number of lactose fermenters and proteolytic anaerobes.

At about this stage in our investigations an observation was made that directed our attention to another phase of the subject. We were fully aware of the fact that dysentery amoebae thrive better in a medium containing starch than in media free from starch and actually ingest starch grains. We found also that trichomonads will ingest starch grains and grow and multiply best in cultures to which this carbohydrate has been added. But not until we placed starch grains in a culture containing balantidia and then watched them under a microscope did we realize how voracious a protozoon can be. These balantidia did not ingest them one by one and at infrequent intervals but swallowed many of them at a time and soon became so gorged with them as to reach twice their normal size. Within 15 minutes 100 per cent of the balantidia had ingested starch grains.

This astonishing observation suggested to us that perhaps starch is used largely as food for the balantidia, and hence, when the host is fed on a high protein diet, these ciliates starve to death. A plan to test this hypothesis was soon developed. This included a study of the correlation between the amount of carbohydrates in the intestine and the number of species and individuals of intestinal protozoa. Guatemala was selected as the scene of our activities since the natives of tropical America are known to harbour large numbers of intestinal protozoa and to live on a diet consisting largely of carbohydrates.

We first had to devise a method of determining the presence and quantity of starch in human faeces. This proved to be a real problem in itself. Then we

collected 100 faecal samples from natives in Guatemala, examined them when fresh so as to record the different species present and the relative numbers of each species, and preserved a sufficient quantity of each specimen with which to determine the starch content. A large amount of time and effort was expended before we were able to evaluate our results. Unfortunately our data indicate no correlation whatever between the presence of starch and the number of species and individual protozoa in the faeces, and I am inclined to believe that it will not be easy to establish such a correlation. This means that other methods will have to be devised before we can hope to explain why modifications of the host resulting from a diet high in carbohydrates favours the growth and multiplication of certain intestinal protozoa.

Various other methods of changing the intestinal contents of the host have been tried but not enough work has been done to warrant discussion except perhaps that of increasing the viscosity of the medium. Various substances were fed to rats infected with trichomonads; the most satisfactory were psyllium seed, agar-agar and gum arabic. The average number of trichomonads per unit in the caecum of rats fed on a normal diet was 392, whereas the number per unit in rats fed on psyllium seed was 55, on agar-agar, 285, and on gum arabic, 7. We also found that the number of balantidia in rats infected with ciliates from a chimpanzee was also less when a viscus condition was set up within the intestine. In normal rats there were counted 347 balantidia per centigram whereas in rats fed on agar-agar the number was 60, on linseed was 92 and on psyllium seed was 34. In no case were all of the trichomonads or all of the balantidia eliminated but these protozoa evidently do not grow and multiply as well when the viscosity of the intestinal contents is increased by changes in the diet.

Intestinal protozoa appear to be able to withstand temperatures below zero centigrade for considerable periods of time without obvious damage, but succumb quickly to high temperatures. This has led to various suggestions involving the use of heat as a method of eliminating these organisms from the host. For the most part temperatures high enough to destroy endoparasitic protozoa are injurious or fatal to the host, but in some cases the host survives after the death of the parasites. For example, the intestine of the large Pacific termite, *Termopsis angusticollis*, is swarming with four different types of flagellates and cannot digest the wood it uses for food without the aid of these protozoa. By subjecting these termites to a temperature of 36° C. for 24 hours all of the flagellates are destroyed, but the hosts are not harmed. This is a temperature that might occur in nature and hence experiments of this type fall within the province of this discussion. The defaunated termites continue to eat wood, but cannot digest it and therefore die in about 3 weeks in the midst of plenty. If these hosts are re-infected they are once more able to utilize the wood they eat and live indefinitely.

Temperature is not the only factor that is capable of modifying termites so as to destroy the intestinal flagellates living in them. Thus if a termite is starved for 6 days it loses one type of flagellate, *Trichonympha,* and if it is starved for 2 days longer it loses a second type of flagellate *Leidyopsis.* Further experiments with oxygen under pressure have demonstrated that the other two types of flagellates, *Trichomonas* and *Streblomastix,* can be removed by this treatment without injury to the termites, but of course no such condition occurs in nature. However, by the use of high temperatures starvation and oxygen under pressure, it has been shown that termites will die in about 3 weeks if all protozoa are eliminated or if all but *Streblomastix* are removed ; that they will die in about 10 weeks if both *Trichonympha* and *Leidyopsis* are removed ; but will live on indefinitely with any combination of flagellates if *Leidyopsis* are present or even with *Leidyopsis* alone. Thus we have proved that the symbiosis involved in this instance is between the termite and *Leidyopsis* and that the other three species are merely hitch-hikers.

Incidentally the most perfect known method of transmission of protozoa from one host to another is exhibited by termite flagellates. The young termites soon after they hatch from the egg are fed by their parents on what is known as proctodeal food. This is excreta of the adults consisting of partially-digested cellulose and many flagellates. One hundred per cent of the young termites are therefore infected soon after they emerge from the egg and are thus supplied with a complete collection of flagellates to digest their food for them.

Blood-inhabiting protozoa have also furnished material for experiments designed to test parasite reactions to host modifications. Today I wish to use the time at my disposal to discuss three methods of treating canary birds that have resulted in obvious changes in infections with the bird malaria parasite *Plasmodium cathemerium.* The first method was to modify the sugar content of the blood by feeding the infected birds with glucose or by treating them with insulin. The clues that led to our experiments came from several sources. Bass, in 1912, found that the addition of sugar to the culture medium was necessary to cultivate malaria parasites outside of the body. In 1913, Bass and Johns reported the cultivation of the organism of human malaria in the blood of a diabetic without the addition of sugar. Certain agents that have been found to be satisfactory as provocatives in bringing on a relapse in malaria such as epinephrin, are known to increase the sugar content of the blood. These facts suggested that the blood stream can be improved as a culture medium for the growth and multiplication of malarial parasites by the addition of sugar. According to this theory, increasing the sugar content of the blood would result in an increase in the number of parasites. *Vice versa,* the blood stream should be rendered less favourable than normal as a habitat for malarial organisms, by decreasing the sugar content. It is not necessary to give details of our experiments here. In general the data indicate that an increase in sugar content renders the blood more favourable for the malaria parasites ; the number of parasites becomes greater and remains so for

a period longer than normal; relapses may occur earlier in the course of the infection and more frequently than normal; and a larger proportion of the birds die as a result of the infection. When treated with insulin the parasite curve is lower than normal throughout the course of the infection. So far as I know, no attempt has been made to modify refractory foreign hosts in this way so that they may become more susceptible, but it seems to me probable that positive results would follow experiments of this nature.

Another method of modifying the host that has been used in the study of malaria is to change the atmospheric humidity and temperature. Malariologists have found that an increase in these factors favours the breeding of mosquitoes and the development of sporozoites within the mosquito, and hence is responsible, at least in part, for epidemics of malaria. We attempted to determine whether such changes modify the vertebrate host so as to effect the development of the asexual and sexual stages in the blood. For this purpose we used canary birds infected with *Plasmodium cathemerium*. In one experiment 12 birds were inoculated each with 50 milligrams of infected blood from a single host. Six were maintained under normal conditions in a room where the temperature was about 70° F. and the relative humidity about 40 per cent. The other 6 were kept in a special room at a temperature of 90° F. and a relative humidity of about 50 per cent. The course of the infections in these 12 birds was carefully followed. The data obtained show that the average number of sexual and asexual parasites was higher in the experimental birds than in the controls and that both types of parasites were present in larger numbers in the blood for a longer period. Thus the higher temperature and humidity appeared to modify the host so that the parasites were able to set up a more severe infection extending over a longer period of time.

A second experiment was performed in which 4 birds were maintained as controls and 7 birds were subjected to an increase in temperature and relative humidity as before. The results confirmed those of the first experiment.

In a third experiment the temperature was increased to 100° F. and the relative humidity to about 90 per cent. Four experimental birds and 3 controls were used. The control birds exhibited normal infections whereas the experimental birds suffered unusually severe infections which resulted in their death.

These experiments add another method of modifying a host, such as is known to occur in nature, which has a favourable influence on the parasite.

Although we have no experimental data of our own to offer at present, it may not be out of place to call attention to the important relation between temperature and the development of malaria parasites in mosquitoes. Low temperatures retard the growth and reproduction of the plasmodia in these insects, and when the temperature reaches a certain degree development ceases entirely. The three species of *Plasmodium* that live in man differ in their reactions to

low temperatures and hence the climatic temperature determines in what regions each species is capable of passing through the sexual cycle within the mosquito and consequently the geographical distribution of the different types of malaria.

Ever since the intermittent nature of malarial fever has been recognized, theories have been proposed to account for this phenomenon. Only recently have definite data on this subject been obtained as a result of experiments on birds.

Canaries infected with *P. cathemerium* were subjected to a reversed daily cycle of exposure to light and darkness. In birds kept in the darkness during the day and in the light during the night the time of sporulation changed within three days from the evening hours to the corresponding morning hours. Birds kept under normal conditions and inoculated with two groups of parasites, one sporulating in the morning and the other in the evening, did not exhibit a periodicity of 12 hours, as might be expected but of 24 hours. Thus the regulatory mechanism appears to be well defined. When infected birds are exposed to the light and darkness for alternate periods of 14 hours, the periodicity changes from 24 to 28 hours, and sporulation occurs mostly at the end of the period of darkness.

Further studies indicate that light in itself is not the factor that regulates periodicity and the time of sporulation; but apparently the feeding habits of the host, which are determined by the light, are closely related to the reproductive cycle of the parasite. Just what this relation is, is still to be discovered. It is interesting to note that when monkeys (*Cebus capucinus*) infected with *P. brasilianum* were subjected to light during the night and darkness during the day for from three to six weeks, the time of sporulation changed so that within two to three weeks sporulation reached its height just after 8 P.M. instead of just after 8 A.M.

Another method of changing the character of the asexual cycle of *P. cathemerium* in canaries has been tested. Blood from infected birds was kept at 5° C. for varying lengths of time and then inoculated into fresh birds. As was to be expected, growth of the parasites at refrigerator temperature was delayed, but when inoculated into fresh canaries the asexual cycle was shortened until sporulation again occurred at the normal time of the day and a periodicity of 24 hours was regained. It is suggested that 'either the asexual cycle represents a genotypic character of the parasites which can be temporarily but not permanently changed by environmental factors, or the cycle is forced on the parasites by the activities of the bird or the diurnal variations in the environment'.

These experiments demonstrate what a remarkable reaction may be stimulated in a parasitic infection by modifications in the behaviour of the host. However, just as in the other experiments described here, the parasitic reaction is maintained only as long as the stimulus is applied. For example, when infected birds are returned to customary day and night conditions and *Balantidium*-infected rats are returned to a diet low in carbohydrates, the host-parasite relations return to normal.

Summary

We have attempted to determine some of the factors that are responsible for host-parasite specificity among protozoa, that is, the infection in nature of a certain species of host by certain species of protozoan parasites. Our plan has been to note the reactions of natural and foreign parasites to modifications in the host. In every case the modifications were brought about by changes in the environment of the host that might easily occur in nature.

Only a beginning has been made but already a number of interesting reactions have been revealed. A carbohydrate diet renders the intestinal contents of certain species of hosts more favourable than normal for some of the intestinal protozoa natural to these hosts. A high-protein diet is correspondingly unfavourable. Of the possible factors that may play a rôle in these results, changes in the hydrogen-ion concentrations seem of little or no importance; the character of the bacteria resulting from these two different kinds of diets may be responsible, or these protozoa may actually require carbohydrate as a food element.

In one case, *Balantidium coli* has been successfully colonized in a foreign host, the rat. This appears to be due solely to a change in diet to one containing a higher percentage of carbohydrates than is normal for this type of host. However, no cysts are known to have been formed in the normally refractory host hence transmission to other individuals of the same species probably does not occur. We cannot, therefore, consider the rat a normal host of this ciliate until we find some factor that will stimulate the formation of cysts.

The reactions of the bird malaria parasites to the modifications cited are of such magnitude as to appear quite striking. Increasing the sugar content of the blood favours the parasite and decreasing it brings about an unfavourable condition. Increasing the temperature and relative humidity of the host's environment also brings about modifications favourable to the parasites.

Changing the periods of light and darkness are followed by definite changes in the course of infections with malaria organisms in canary birds but whether this is due to light rays, food, the muscular activity of the bird, or to some other factor is still to be determined.

At least one result of practical application has developed out of these experiments, that is, the use of a high protein diet for the elimination of certain intestinal protozoa of man. Patients suffering from digestive disturbances due to intestinal protozoa are not numerous but those who are so unfortunate as to be infected welcome with fervor the relief this type of treatment brings to them.

In conclusion, I wish to emphasize the desirability of investigations directed towards *rendering hosts more favourable as habitats* for parasitic protozoa by modifications due to known factors. By this procedure valuable data may be obtained that might not appear when efforts are aimed solely at the elimination of the organisms.

NEW LIGHT ON THE LIFE-CYCLE OF THE MALARIA PARASITE

(Reprinted from the 'Leading Articles' Section of *The Lancet*, No. 13, Vol. 1, 1937, 764.)

It has long been a source of interest to the biologist and of chagrin to the clinician that the malaria parasite, when banished from the blood stream by quinine, is able somewhere to maintain its existence. That the parasiticidal effect of quinine has been at the same time so complete and yet so futile is one of the notable mysteries of clinical medicine. If it is true that the sporozoites injected by the mosquito complete their life-cycle in the cells of the circulating blood, then the infection should be scotched by the ingestion of quinine sufficient to maintain a lethal concentration in the blood stream. And yet a patient who has taken a gramme of quinine daily before and during infection by mosquito bites, and for every day throughout the incubation period, develops an attack of malaria precisely as if no drug had been given. The life-history of the parasite in the vertebral host cannot then be as obvious as all that; for, though Schaudinn described the penetration of sporozoites into red blood corpuscles, no one it seems has succeeded in repeating his observations.

Some years ago Lieutenant-Colonel S. P. James made the suggestion[1] that the sporozoites, when they are injected by the mosquito, may be carried to reticulo-endothelial cells of the lungs and other organs in which they complete their asexual cycle of growth and sporulation—a process already observed in the bird parasite, halteridium. Colonel James has now confirmed this suggestion for a malaria parasite itself—not in man, but in the domestic fowl. At a laboratory meeting of the Royal Society of Tropical Medicine and Hygiene on March 18th, he demonstrated the life-history of the *Plasmodium gallinaceum* as worked out by Mr. P. Tate, Ph.D., and himself at the Molteno Institute in Cambridge. Using this avian strain, brought from Ceylon by Professor Brumpt of Paris, James and Tate have witnessed a cycle of development occurring, not in red blood-cells, but in the reticulo-endothelial cells of the spleen, liver, kidneys, and particularly the endothelial cells lining the capillaries of the brain. In chickens which had died of a malarial attack, despite the destruction by quinine of all the parasites in the peripheral blood, they found the capillaries of the brain blocked with masses of schizonts, the result being death from paralysis and coma. Those who were able to study the slides submitted at the laboratory meeting were left in no doubt that in avian malaria there is a schizogenous cycle of development occurring in reticulo-endothelial cells. Parallel observations have been made for the blood-forming cells by two Chicago workers[2] and for the reticulo-endothelial cells by

RAFFAELE[3] in Rome. JAMES and TATE have observed the entry of the parasite into blood-forming cells, and every stage in their growth and multiplication until the merozoites burst the cell and are ready to enter other endothelial cells and red blood-cells, to begin the cycle anew.

But while this is true of certain avian parasites it may not apply to those of man, although there is some justification for thinking that it may, if, as we believe, it is a fact that the three fundamental discoveries in malaria—associated with the names of ROSS, McCALLUM, and ROEHL—were made first in birds and only afterwards in man. If the analogy should prove true, the discovery affords no short cut to treatment. But any light shed on the life-history of malaria parasites should indicate the direction in which successful treatment lies ; for a drug which circulates only in the blood stream cannot effectively attack extrahaemic parasites. In the meantime the biologist may have to revise the classification of plasmodia in view of their affinity for tissue cells.

1. *Proc. R. Acad. Med., Amst.* 1931, **34**, 1424.
2. Huff, C. G. and Bloom, W. (1935). *J. Infect. Dis.* **57**, 315.
3. Raffaele, G. (1936). *Riv. Malariol.* **15**, 309, 318.

ABSTRACTS

Researches into sterility of cows in South Africa. The influence of (i) **Dry rations,** (ii) **Lack of exercise, and** (iii) **Lack of sunlight on reproduction in beef heifers and cows.** QUINLAN, J., and ROUX, LUCIEN, L. (*Ond. J. Vet. Sci. An. Hus.* **6**, 2, 719.)

It has been mentioned in the literature that factors like lack of exercise, lack of sunlight, lack of green feed and high condition have been associated with low fertility in cattle but such conclusions have been based on circumstantial evidence without experimental proof. The object of the work undertaken by the authors was to collect and record data on the behaviour of the genital tract of twenty-seven beef (Sussex-Afrikandar) heifers and cows when placed in an environment enforcing the above-mentioned factors, over a period of six years which included four calvings. Usually insufficiency of light and exercise are associated with unhealthy stabling and general bad management, but in this experiment these associated hygienic factors could be ruled out.

When a dry ration consisting of maize, wheaten bran and teff hay was fed for nine months and silage was added to the ration during the remaining three months of the year, very satisfactory results were obtained. The ration induced satisfactory growth of young heifers and maintained mature animals in good condition. Sexual maturity was reached before the age of 24 months and sexual activity and reproduction were in no way impaired.

High condition caused no ill-effects either upon general health or reproductive processes but under conditions of restricted sunlight or exercise, breeding was delayed until the high conditioned heifers were thirty-five months of age. Cows which maintained a higher condition throughout the periods of gestation, lactation and rest usually produced calves which were small and light in weight.

The restriction of sunlight and exercise in no way detrimentally affected the health, growth and vigour of heifers and cows, being fed dry rations. The onset of maturity was not delayed and it appeared to shorten rather than to lengthen the period of inactivity between calving and the first subsequent oestrus. About 75 per cent of dioestral cycles fell between eighteen and twenty-three days and the animals receiving an abundance of sunlight and exercise experienced a larger number of abnormally long dioestrus cycles. It appeared that as the ages of the animals advanced under the special environmental conditions enforced, the percentage of dioestrus cycles between eighteen and twenty-three days decreased and the period of inactivity appeared to shorten as the ages of the cows advanced between three and six years. The length of gestation period which in 81 per cent of the cases fell between 276 and 286 days, was not altered by limiting sunlight and exercise or with advanced age and there was no difference between the gestation periods of males or females.

Unrestricted sunlight and exercise resulted in fewer services being required to establish pregnancy. Failure to conceive after the first and second service could not be considered exceptional and that appeared to be the case especially in young animals. The number of services required to establish fertility decreased as the ages of cows advanced. Unrestricted sunlight and exercise did not reflect any advantage upon the weight of calves produced. It was observed that the change of the bull greatly influenced the birth-weight of the calves.

It is highly probable, in view of the results recorded in this paper, that some secondary concurrent factors are necessary to cause that deviation in sex-physiology which produces difficult breeding in cattle fed on dry rations and showing obesity or those which are housed under conditions enforcing restrictions of exercise and sunlight. [R. L. K.]

The treatment of ascariasis in chickens. LEVINE, P. P. (*The Cornell Veterinarian* **26**, 2, 120-127.)

ASCARIDS are the most important helminths infesting poultry. Beach and Freeborn (1922) were the first to advocate the use of tobacco dust in the mash for the expulsion of the large roundworms in chickens. They mixed tobacco dust with a nicotine content of 1½ to 2 per cent with dry mash in the proportion of two parts per hundred and fed the mixture to infested birds for one to four weeks. The latter author (1923) removed nearly all the roundworms from infested birds in this way, without any ill-effects to the treated birds. Graybill and Beach (1925), reported that the efficacy of the above treatment for the individual birds ranged from 40 to 100 per cent and for the group as a whole 76 per cent. In 1927 Beach and Freeborn observed that as the nicotine content of tobacco was variable and the mash was not eaten readily by the infested birds, treatment with tobacco dust in most cases was not reliable. They, however, recommended dosing birds individually with capsules of Black Leaf 40 and Lloyd's Alkaloidal Reagent. Carpenter (1926) tested the efficacy of these capsules on 350 birds and found it to be 96·8 per cent efficient. He further considered it advisable to feed tobacco dust containing 1·5 per cent nicotine in proportion of two per cent of the mash for three weeks after giving the capsules.

The author carried out experiments to evaluate the tobacco dust treatment for controlling roundworms in poultry. The dust used was a commercial product with nicotine content of 1·78 per cent by weight. He concluded that the tobacco dust fed to chickens continuously from the day of hatching in concentrations of 2, 4, 6, and 8 per cent in a dry mash did not prevent the hatching of embryonated eggs of *A. lineata* and the invasion of the intestinal mucosa by the larvae. Two per cent concentration of tobacco was not toxic for chicks, but 40 per cent of the chicks fed on 8 per cent concentration died of poisoning. He found that immature worms are less susceptible to tobacco treatment than adults.

A nicotine compound (Black Leaf Powder) given in single dose removed all the worms from forty-five pullets infested with adult worms. However, the powder may not be 100 per cent efficient under all conditions. Quarter pound powder was mixed in 5 lbs. mash for every hundred birds. Feed was withdrawn from the birds the evening before treatment. The following morning mash was given which they readily took.

The author maintains that continuous feeding of tobacco dust in mash to prevent ascarid infestation is ineffective. The feeding of the dust for four weeks to older birds was less effective than a single treatment with nicotine powder. [H. D. S.]

The use of nitric acid in the serological diagnosing of cattle trypanosomiasis. JONES, E. R. (*Vet. Rec.* **48**, 602-605.)

PRECIPITATION of excess of euglobulin produced in trypanosomiasis has been noted by this author by employing twenty different chemicals. Eight hundred and fifty-seven tests, based on the theory of precipitation of serum euglobulin excess in trypanosomiasis, were carried out to demonstrate the specific nature of reaction yielded by the use of nitric acid. Tests were performed with the technique employed by Bennett and Kenny (1928—*Jour. Comp. Path & Therap.*, **41**, 341) *i.e.*, one drop of clear serum added to 1 c.c. of the reagent; the drops being added with a Dreyer's pipette. This gave an equivalent dilution of 1 : 25. Different concentrations of nitric acid (1·8 per cent, 1·5 per cent and 1·3 per cent W/V HNO_3) were used and the time reading of reactions was noted. Of 249 tests in which the reaction was allowed to proceed to exactly one hour the following result was noted :—With 1·8 per cent, forty-five per cent of the cases gave positive reaction ; with 1·5 per cent, and 1·3 per cent, 21 per cent gave doubtful reaction ; while 34 per cent were negative with latter concentrations. One hundred and thirty tests were carried out in which the reaction was noted immediately after 1·8 per cent W/V HNO_3 was added and it was observed that 51 per cent of the cases gave positive while 49 per cent gave negative or doubtful reaction.

It is indicated that application of nitric acid as a serological test is a modification of the xanthoproetic reaction for proteins. The precipitate produced in mercuric chloride test is in the form of metaprotein, while those with nitric acid, according to the author, is euglobulin itself.

It has been pointed out that the reaction is seldom given in the early stages of infection when trypanosomes are present in the blood—in which case direct blood examination suffices, but it was conclusive afterwards in chronic or "latent" infections when parasites could not be detected in the blood. It has been suggested by the author that further tests would have to be carried out to determine (*a*) the period of infection before the test becomes positive, and (*b*) the specificity, if any, of the test in relation to other diseases.

Addition of 0·025 per cent of phenol to the serum as a preservative did not alter the reaction which could still be obtained seven to eight days after the serum had been collected. Affected cases used by the author in his series of tests were known to be infected with trypanosomes belonging to *Vivax*, *Congolense* and *Brucei* groups. [H. N. R.]

Sheep nutrition I. Measurements of the appetites of sheep on typical winter rations, together with a critical study of the sheep-feeding standards. WOODMAN, H. E., EVANS, R. E. and EDEN, A. (*J. Agri. Sci.* **27**, 191-211.)

IN three successive years, during the winter, feeding experiments were carried out with sheep kept out-of-doors on typical winter rations as lucerne hay, hay, rye grass-sainfoin hay, swedes, marrow-stem kale and potatoes supplemented with concentrates usually fed in farms. Nine, five and seven wethers were used in the respective years ranging in age from three months to two and a half years and in live-weight from 60 to 200 lbs. During the experimental periods the live-weights as well as daily consumption of dry matter and starch equivalent were recorded.

Appetites were found to differ widely with diet and individuality. Even in the same animal with the same ration considerable day to day fluctuations were noticed. Variations in appetite ranged from 69·1 per cent to 108 per cent of the values predicted by Professor T. B. Wood. These latter were found to be uniformly too high, thus confirming the results of Professor Watson and co-workers at Oxford. It is suggested that Professor Wood's values should be multiplied throughout by the factor 0·85 to obtain reasonable measures of the appetites of sheep at different live-weights.

From the results obtained in the feeding trials it has been found that the recent standard of 1·26 lbs. of starch equivalent per day was more reliable than the old one (Armsby standard) of 0·74 lb. starch equivalent per day for the maintenance requirement of 100 lbs. sheep. The higher figure enabled the prediction of live-weight increment with much greater precision. Recent work on the energy metabolism of sheep have also given values in agreement with the higher figure.

A table embodying a revision of Professor Wood's feeding standards for sheep is included in the paper. [T. S. K.]

Sheep nutrition II. Determination of the amounts of grass consumed by sheep on pasturage of varying quality. WOODMAN, H. E., EVANS, R. E. and EDEN, A. (*J. Agri. Sci.* **27**, 212-223.)

IN order to determine the amounts of grass consumed by sheep on pasturage grazing trials were conducted on the light-land pasture at Cambridge. The main plot was divided by means of hurdles into five subplots. Two groups of four and five wethers each were used for the grazing trials. The sheep were passed from plot to plot in succession, the animals being moved on at a rate dictated by the necessity of always having an abundance of grass available for grazing.

The average daily output of faeces by the sheep on pasture was determined and during this period the digestibility of the same herbage was found out by independent digestion trials on other sheep. By a simple calculation the grass consumption of the animals on pasture was arrived at.

Sheep grazing on very good leafy pasture ate on an average 5 to 6 lbs. of dry matter per day which is about 1·3 lbs. in excess of what they would consume on ordinary winter rations fed *ad lib.* When the quality of the pasture deteriorated owing to unfavourable weather conditions the consumption also diminished markedly. However, even under such unfavourable conditions the dry matter consumption when grazing was greater than on winter ration by 0·41 lb. and 0·72 lb. per day in two separate experiments, the variation depending on the degree of deterioration.

It is clear from the results of the experiments that sheep consume a bigger ration, in terms of lbs. dry matter, when on pasture than when subsisting out-of-doors on the usual winter diet. The consumption is highest with young leafy pasture and diminishes with deterioration in its quality. [T. S. K.]

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